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ALDH Activity Indicates Increased Tumorigenic Cells, But Not Cancer Stem Cells, in Prostate Cancer Cell Lines

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Abstract. *Background: Cancer stem cells (CSCs) have been shown to be a small stem cell-like cell population which appears to drive tumorigenesis, tumor recurrence and metastasis. Thus, identification and characterization of CSCs may be critical to defining effective anticancer therapies. In prostate cancer (PCa), the CD44$^+$ cell population appears to have stem cell-like properties including being tumorigenic. The enzyme aldehyde dehydrogenase (ALDH) has been found to identify hematopoietic stem cells and our aim was to determine the utility of ALDH activity and CD44 in identifying PCa stem cell-like cells in PCa cell lines. Materials and Methods: LNCaP cells and PC-3 cells were sorted based on their expression of CD44 and ALDH activity. The cell populations were investigated using colony-forming assays, invasion assays, sphere formation experiments in a non-adherent environment and 3-D Matrigel matrix culture to observe the in vitro stem-cell like properties. Different sorted cell populations were injected subcutaneously into NOD/SCID mice to determine the corresponding tumorigenic capacities. Results: ALDH^{hi} CD44$^+$ cells exhibit a higher proliferative, clonogenic and metastatic capacity in vitro and demonstrate higher tumorigencity capacity in vivo than did ALDH^{lo} CD44$^-$ cells. The tumors recapitulated the population of the original cell line. However, ALDHlo CD44$^-$ cells were able to develop tumors, albeit with longer latency periods. Conclusion: ALDH activity and CD44 do not appear to identify PCa stem cells; however, they do indicate increased tumorigenic and metastatic potential, indicating their potential importance for further exploration.*

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Prostate cancer (PCa) is the third leading cause of cancer-related deaths among men in America (1). In 2006, an estimated 27,000 American men died of prostate cancer and an estimated 230,000 new cases were diagnosed (1). The concept of the 'cancer stem cell' (CSCs) was introduced more than 50 years ago when it was recognized that only a small proportion of cells (0.01%-1%) in tumor isolates are clonogenic and extensively proliferative *in vitro* and *in vivo* (2, 3), indicating that these cells might represent tumor stem cells. The CSC hypothesis was recently revived following the development of novel methods for identification, purification and characterization of normal stem cells. The most stringent definition is that a CSC should be a cell that at the single-cell level can reconstitute a tumor that is identical to the parental tumor and that can be serially xenotransplanted indefinitely (4). CSCs appear to be highly tumorigenic and may drive tumor proliferation, differentiation and maintenance, thus the goal of cancer therapy study will be to identify, characterize and eliminate this small population. It is hypothesized that PCa, like other types of cancer, might arise from stem or progenitor cells (5). Yet the study of PCa CSCs has been hindered by a lack of well-established cell surface markers.

CD44, which is expressed in most prostate basal cells, is an adhesion molecule with multiple signaling functions (6). It has been reported to play an important role in tumor migration and metastasis in PCa and breast cancer cells (7). CD44 expression is found in stem/progenitor cells for multiple tissues including hematopoietic stem cells (8), mesenchymal stem cells (9), neural stem/progenitor cells (10), astrocyte precursor cells (11) and mammary stem/progenitor cells (12). CD44 has been shown to be a possible marker of PCa CSCs. Specifically, CD44$^+$ PCa cells obtained from xenografts of human tumors were shown to have increased tumorigenic, clonogenic, and metastatic potential compared to CD44$^-$ PCa cells (13). Furthermore, CD44$^+$ CD133$^+$ integrin $\alpha2\beta1^{hi}$ PCa cells represented the tumorigenic cells in primary cell cultures derived from PCa patients (14).

Although primary cell culture studies have the strength of providing the original features of the tissue of derivation, they also have limitations, such as difficulty in obtaining biopsy material, short lifespan of cells and the very small population of potential stem cells due to the small amount of material that can typically be obtained. Therefore, identification and characterization of CSCs in established cell lines may provide important tools for exploring the biology of CSCs. Along these lines, 'side population' (SP) analyses (15, 16) and cell surface markers such as CD133 (17, 18) have demonstrated potential for the identification of CSCs in established cancer cell cultures.

Aldehyde dehydrogenase (ALDH) is an enzyme that is expressed in liver and is required for the conversion of retinol (vitamin A) to retinoic acid. Recently, detection of ALDH activity has been investigated as a marker of hematopoietic stem/progenitor cell (6-9) cells and it was reported that high ALDH activity correlates with the stem/progenitor cell state (10-12). ALDH is also a key regulator of hematopoietic stem cell differentiation as demonstrated by the observation that inhibition of ALDH delays the differentiation of human hematopoietic stem cells (13).

In the current study, we hypothesized that ALDH activity is a marker of stem cells in PCa cell lines. We investigated the *in vitro* and *in vivo* proliferative behavior of cells with high ALDH expression ($ALDH^{hi}$), with CD44 as a co-marker to characterize different cell populations.

Materials and Methods

Cell lines. LNCaP and PC-3 human PCa cells (obtained from the American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI-1640 containing penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (FBS) at 37˚C in incubator with 5% CO_2.

Isolation and flow cytometric analysis. Cells were trypsinized, washed with phosphate buffered saline (PBS) and stained with Aldefluor reagent (StemCo Biomedical, Durham, NC, USA). Aldefluor substrate was added to 1×10^6 cells/ml suspended in Aldefluor assay buffer and incubated at 37˚C for 30-60 minutes. Meanwhile, 5 μl of diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor, were added to 0.5 ml of Aldefluor-stained cells as a negative control. Cells were co-stained with Allophycocyanin (APC) anti-human CD44 antibody (BD Pharmingen, San Diego, CA, USA). The expression of surface markers was analyzed and sorted with FACS using a FACS Vantage SE cell sorter (BD Pharmingen).

Colony formation assay. The colony formation assay was performed by culture of isolated cell populations on collagen I 6-well plates (BD Biosciences), with 1,000 single cell suspension in each well. Colonies were enumerated by microscopy after incubation at 37˚C for 7-10 days until the colonies were formed. Colony diameters larger than 75 μm or colonies numbering more than 50 cells were counted as 1 positive colony.

Matrigel invasion assay. Matrigel invasion chamber with 8 μm pore size inserts pre-coated with Matrigel Basement Membrane Matrix was used in accordance with the manufacturer's instructions (BD Biosciences). Isolated PC-3 cell populations were plated into triplicates at a density of 1×10^4 cells/well. The membrane was stained after 48 hours and cell numbers plotted as a percentage of invasion through the Matrigel matrix and membrane relative to the migration through the control membrane.

Cell differentiation in Matrigel. PC-3 cells were trypsinized and 5,000 cells were mixed with Matrigel Basement Membrane Matrix (BD Biosciences). The mixture was allowed to solidify in a 12-well plate at 37˚C and then were cultured in regular medium.

Serum-free culture. Isolated PC-3 cell populations were seeded at a density of 3,000 cells/well into low-attachment 6-well plates (BD Biosciences) and at a density of 500 cells /well into regular 96-well plates, both containing serum-free medium supplemented with epidermal growth factor (EGF) 100 ng/ml. After a 7 -day incubation at 37˚C, the morphology of the cells was assayed and pictures were taken under light microscopy.

Transplantation. After isolation by flow cytometry, PC-3 cell populations were subcutaneously injected into 6- to 8-week-old male NOD/SCID (Jackson Labs, USA) mice at a series of 100, 1,000 and 10,000 cells in 0.1 ml regular medium mixed with a 1:1 ratio of matrigel (BD Biosciences). Mice were monitored daily to observe palpable tumors. Tumor sizes were measured with calipers twice a week after palpable tumors appeared. The mice were euthanized 10 weeks after tumor cells injection and tumors were excised and dissociated to single-cell suspensions that were analyzed by FACS.

Statistics. The data were described using the mean value of each group and standard deviation (SD). Data were assessed by one-way ANOVA. Statistical significance was determined at $p<0.05$.

Results

ALDH activity and CD44 are highly expressed in PCa cell lines. Since elevated ALDH activity has been shown to be a marker of long-term reconstituting human hematopoietic stem cells (19, 20), and CD44 has been identified as a surface marker in PCa stem cell studies (4, 14), we identified the distribution of ALDH and CD44 expression in human PCa cell lines. The component of $ALDH^{hi}$ $CD44^+$ subpopulation varied in different PCa cell lines. LNCaP and PC-3 cells were chosen to be sorted. In LNCaP cells, 4.7% of the population was $ALDH^{hi}$, whereas CD44 expression was not detectable in this cell line (Figure 1A). In contrast, the PC-3 cell population consisted of 18% $ALDH^{hi}$ and 55% $CD44^+$ cells and 7.2% that were positive for both markers (Figure1B).

$ALDH^{hi}$ $CD44^+$ cells exhibit a high proliferative, clonogenic and metastatic capacity in vitro. To determine whether ALDH indicates CSC-like properties in LNCaP and PC-3

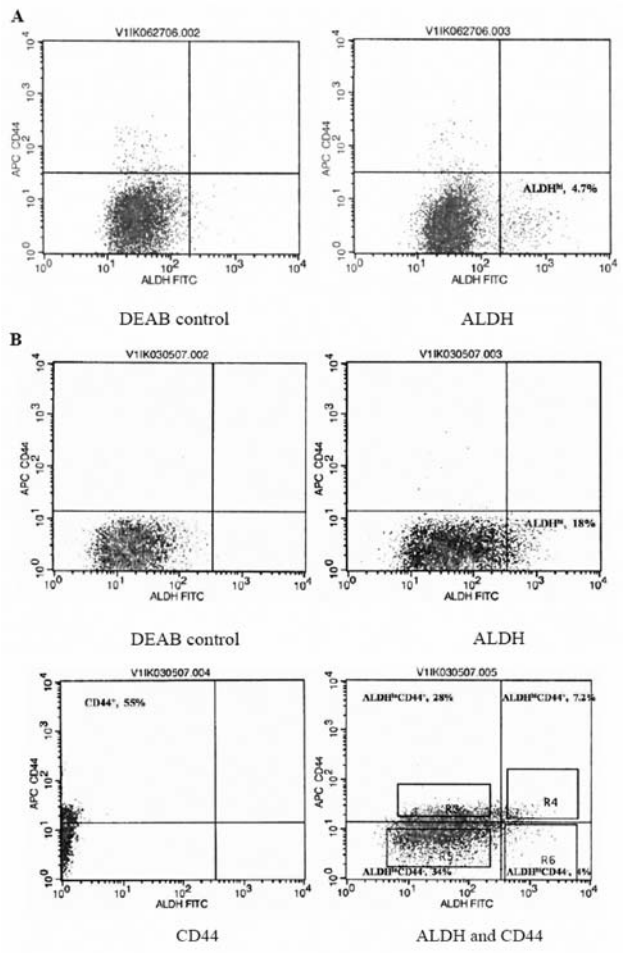

Figure 1. *Flow cytometric analysis of ALDH activity and CD44 expression in PCa cells. Cells were stained with both ALDH substrate and CD44 antibody, with DEAB staining as a negative control for ALDH expression. Dead cells were eliminated from the analysis by PE staining. The median percentage of each population is given. A, LNCaP cells were incubated with ALDH substrate and DEAB to select ALDH^{hi} cells from ALDH^{lo} cells. B, PC-3 cells were analyzed for ALDH and CD44 expressions.*

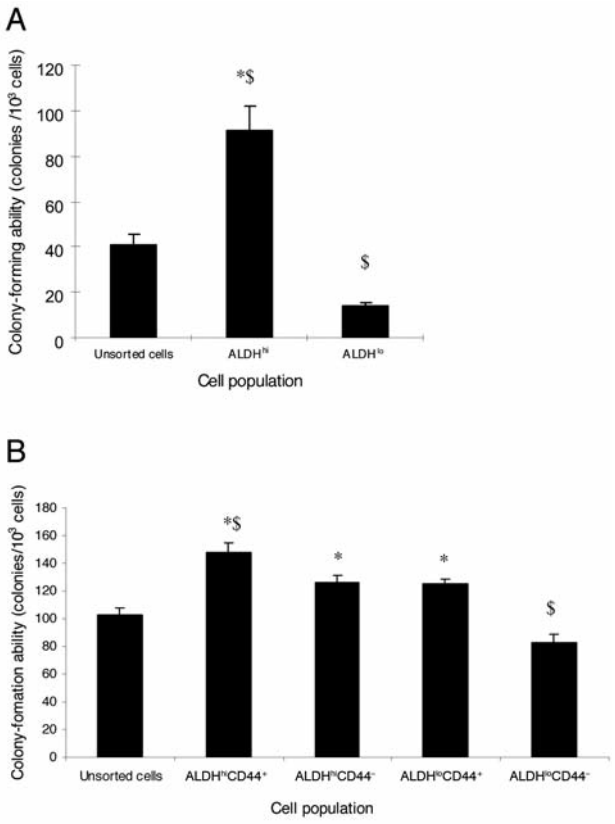

Figure 2. *In vitro progenitor capacity of isolated ALDH and CD44 populations. A: 1,000 ALDH^{hi} and ALDH^{lo} LNCaP cells were plated in regular media on collagen-coated plates and cultured for 7-10 days. The cell colonies were then enumerated under a microscope. B: Four populations of PC-3 cells, ALDH^{hi} CD44^{+}, ALDH^{hi} CD44^{-}, ALDH^{lo} CD44^{+}, and ALDHlo CD44^{-}, were cultured and enumerated for colony-forming assay after sorting with flow cytometry.*

cells, the PCa cells were sorted using flow cytometry and cultured in regular media on collagen-coated plates to observe their colony forming ability. ALDH^{hi} LNCaP cells showed enhanced (p<0.01) colony production compared to the parental unsorted population and the ALDH^{lo} cells (Figure 2A). Additionally, the ALDH^{lo} population had a lower colony-forming ability than did the unsorted population (Figure 2A, p<0.05), indicating that removal of the ALDH^{hi} cells from the unsorted population reduced its clonogenic ability. In the case of PC-3 cells, ALDH^{hi} CD44^{+}, ALDH^{hi} CD44^{-} and ALDH^{lo} CD44^{+} cells all had higher colony forming ability than ALDH^{lo} CD44^{-} populations (Figure 2B, p<0.05). Of note, the ALDH^{hi} CD44^{+} had the highest colony forming ability. Furthermore, ALDH^{lo} CD44^{-} population had a lower colony-forming ability than did the unsorted cells. These results indicate that (i) ALDH^{hi} CD44^{+} cells possess a high clonogenic function and (ii) that ALDH confers clonogenic potential. Although these results are suggestive of ALDH being associated with CSC-like properties, it is recognized that *in vitro* clonogenic progenitor production does not guarantee repopulating function in immune-deficient mice (10).

To study the metastatic potential of cells with ALDH and CD44 markers, sorted PC-3 cell populations were subjected to an invasion assay. ALDHhi CD44^{+}, ALDHhi CD44^{-}, and ALDHlo CD44^{+} populations each were more invasive than unsorted and ALDH^{lo} CD44^{-} cells (Figure 3A, p<0.05). Furthermore, the double-positive cells (namely ALDH^{hi} CD44^{+} PC-3 cells) had the highest level of invasion, suggesting that both ALDH and CD44 markers are associated with factors that contribute to invasive ability.

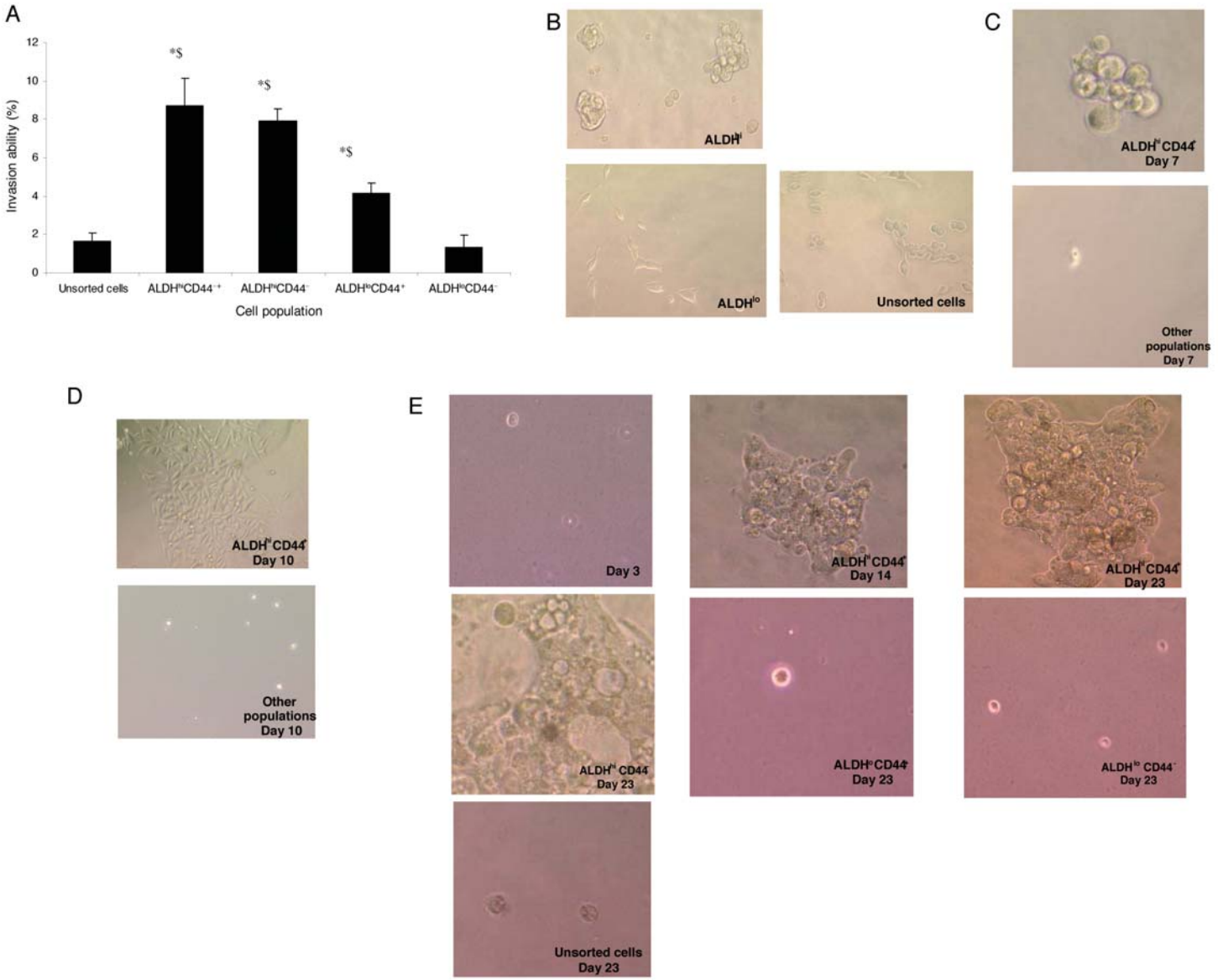

Figure 3. *Influence of ALDH activity on invasive ability, morphology and adhesion-free growth of PCa cell lines. A: The sorted PC-3 cell populations were plated into Matrigel invasion chambers and their invasion abilities were analyzed. *Statistically significant difference compared with ALDHlo (LNCaP cells), and ALDHlo CD44^{-} (PC-3 cells) population respectively; $^{\$}$statistically significant difference compared with the unsorted cells. B: LNCaP populations were cultured in regular 96-well plates and the cell morphology was recorded 10 days later. C: PC-3 cell populations were cultured in serum-free medium containing EGF in low-attachment 6-well plates. D: PC-3 cell populations were cultured in serum-free medium containing EGF in regular 96-well plates. E: The isolated PC-3 cell populations were mixed with Matrigel matrix and cultured for 23 days. The cell morphology was observed under microscope every 2 days.*

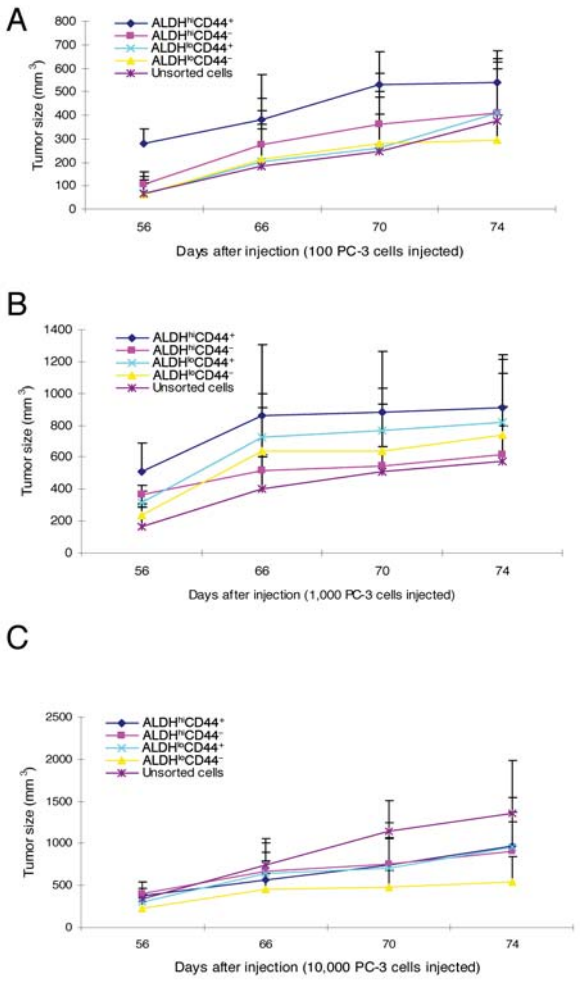

Figure 4. *In vivo tumorigenicity of subcutaneously injected sorted PC-3 cells. After isolation by flow cytometry, 0.1 ml of PC-3 cells were subcutaneously injected into NOD/SCID mice, with 100, 1,000, 10,000 cells and mixed at a 1:1 ratio with matrigel. Mice were monitored daily to observe palpable tumor. Tumor sizes were measured with calipers and calculated with the formula V=length×width^2×π/6. Mice were injected with 100 (A), 1,000 (B) and 10,000 (C) sorted cells.*

In order to further evaluate the tumorigenic property of ALDHhi cells, we evaluated the morphological difference among the sorted cell populations. The appearance of non-adherent spherical clusters of cells in serum-free medium has been a major *in vitro* advance in stem cell culture (22) that allows for the cultivation of CSCs from brain (23) and breast tumors (24). Interestingly, even cultured in regular 96-well plate with medium containing serum, the ALDH^{hi} LNCaP cells demonstrated a significant production of spheroid colonies; whereas the ALDH^{lo} LNCaP cells did not form spheres or colonies but only stromal-appearing individual cells. Furthermore, the morphology of cultures derived from the unsorted control cells consisted of a mixture of that seen in both the ALDH^{hi} and ALDH^{lo} populations (Figure 3B). In contrast, the PC-3 cell populations did not exhibit much difference in morphology when cultured in regular 96-well plates. The sorted PC-3 cells were seeded into low-attachment plates with serum-free medium supplemented with EGF. Only the ALDH^{hi} CD44^{+} cells survived and formed spheroid colonies (Figure 3C), whereas the other populations died after 1 week. The cells were also plated into regular 96-well plates in serum-free medium and only ALDH^{hi} CD44^{+} formed large colonies (Figure 3D), while the other populations maintained single-cell states and died about 10 days later. These results demonstrate that the combination of ALDH and CD44 expression confers the ability of attachment-free growth.

Matrigel matrix 3-D culture has been shown to be an effective system to encourage stem cell growth (25). Additionally, the Matrigel culture system can be used to demonstrate differences in the morphological differentiation between normal prostatic epithelial cells and PCa cells (26). Therefore, to determine if ALDH^{hi} cells have any clonogenic properties in Matrigel matrix, the sorted PC-3 cell populations were embedded and cultured in Matrigel matrix. Both ALDH^{hi} CD44^{+} and ALDH^{hi} CD44^{-} populations proliferated and differentiated into colonies in Matrigel matrix (Figure 3E). The cells remained as single cells on the third day, and then formed colonies on day 14 that continued to expand to the end of the study on day 23. In contrast, the ALDH^{lo} CD44^{+}, ALDH^{lo} CD44^{-} and the unsorted control cells did not form colonies, but maintained the single-cell morphology and gradually stopped growing and died within 2 weeks. These results indicate that expression of ALDH, as opposed to that of CD44, indicates the clonogenic potential of cells in a simulated 3-D growth environment.

ALDH^{hi} CD44^{+} cells demonstrate in vivo tumorigenicity. To assess whether ALDH^{hi} CD44^{+} cells possess tumorigenic properties *in vivo*, we performed subcutaneous injection of sorted PC-3 cells at different doses (100, 1,000 and 10,000 cells per injection) into NOD/SCID mice. When cells were injected at 100 or 1,000 cells per injection, the ALDH^{hi} CD44^{+} cells formed larger tumors with shorter latency periods than in the mice injected with the other populations of sorted cells and the unsorted cells (Figure 3A and B and Table I). In contrast, when cells were injected at 10,000 cells per injection, the unsorted cells formed the largest tumors; however the ALDH^{hi} CD44^{+} cells still had the shortest latency (Figure 3C and Table I). Furthermore, the ALDH^{lo} CD44^{-} cells formed the smallest tumors and there was no difference in tumor growth among the other cell populations. These results indicate that the presence of ALDH and CD44 both confer a growth advantage, which is additive, at low cell densities. However, at higher cell densities, this growth advantage appears to be lost.

Table I. *Tumorigenicity of subcutaneously injected PC-3 cells.*

PC-3 cells injected[a]	Population	Tumor incidence[b]	Latency (days)[c]
100			
	$ALDH^{hi}CD44^{+}$	5/5	50±2
	$ALDH^{hi}$ $CD44^{-}$	5/5	52±2
	$ALDH^{lo}CD44^{+}$	4/5	54±3
	$ALDH^{lo}CD44^{-}$	5/5	53±2
	Unsorted cells	2/5	51±3
1000			
	$ALDH^{hi}CD44^{+}$	5/5	48±3
	$ALDH^{hi}CD44^{-}$	5/5	49±2
	$ALDH^{lo}CD44^{+}$	4/5	53±2
	$ALDH^{lo}CD44^{-}$	4/5	53.5±1.5
	Unsorted cells	4/5	53.5±1.5
10000			
	$ALDH^{hi}CD44^{+}$	5/5	45±2
	$ALDH^{hi}CD44^{-}$	5/5	47±2.5
	$ALDH^{lo}CD44^{+}$	4/5	51±2
	$ALDH^{lo}CD44^{-}$	3/5	53±3
	Unsorted cells	2/5	55.5±2.5

[a]PC-3 cells were mixed at a 1:1 ratio with matrigel subcutaneously injected into NOD/SCID mice on the back of the mice. [b]The number of tumors/ number of injections. [c]From tumor cell injection to the appearance of a palpable tumor (median±SD). *$p<0.05$ compared with the corresponding $ALDH^{lo}CD44^{-}$ and unsorted cell injections.

A feature of stem cells is their ability to recapitulate the cell population from which they are derived. To determine if the tumors that formed from the sorted cell populations recapitulated the original unsorted population, single-cell suspensions of tumors were subjected to flow cytometry. The cell population distribution of all the tumors derived from the different sorted cell populations was similar to the original unsorted population (Table II). This indicates that all the populations, including $ALDH^{lo}$ cells and $CD44^{-}$ populations, contained cells that are able to differentiate.

Discussion

In the present investigation, we determined that ALDH expression in PCa cell lines is associated with increased colony-forming ability, tumor growth and invasive properties. However, ALDH does not appear to specifically identify CSCs in cell lines based on the observation that $ALDH^{lo}$ cells were able to develop tumors and recapitulate the spectra of the unsorted cell population, albeit at a lesser magnitude than ALDH-positive cells. It is important to point out that these studies only evaluated cell lines and not tumor tissues, thus, these results do not address whether ALDH is a marker of CSCs in primary tumors; however, they provide important information regarding the role of ALDH in PCa cell lines.

Table II. *FACS analysis of the tumor cells dissociated from tumors induced by injection with different PC-3 cell populations.*

PC-3 cell population injected	$ALDH^{hi}$	$CD44^{+}$	$ALDH^{hi}CD44^{+}$
$ALDH^{hi}CD44+$	1.64±1.23%	58.59±12.35%	2.58±2.32%
$ALDH^{hi}CD44^{-}$	2.57±1.67%	30.71±9.83%	3.16±1.57%
$ALDH^{lo}CD44^{+}$	3.16±2.34%	17.05±6.92%	2.88±2.93%
$ALDH^{lo}CD44^{-}$	0.55±1.03%	30.46±19.32%	1.47±1.22%

Data shown are the mean±SD.

Several cell surface proteins have been identified as markers of CSCs in a variety of cancer types such as hematopoietic disease (20, 27, 28), breast cancer, hepatocellular carcinoma (18), brain tumor (23, 29, 30), lung cancer (31) and melanoma (32). In terms of PCa, CD133 (26), CD44 (14) and $\alpha2\beta1$ (14) have been reported as CSCS markers.

ALDH is a family of enzymes involved in the metabolism of aldehydes to their corresponding carboxylic acids. In the liver, cytosolic ALDH contributes to the biosynthesis of retinoic acid from retinol (vitamin A). Corti *et al.* reported that SSC (side scatter)$^{lo}ALDH^{br}$ (=$ALDH^{hi}$) neural cells are capable of self-renewal and are able to generate new neurospheres and neuroepithelial stem-like cells; furthermore, these cells are multipotent, differentiating both in neurons and macroglia (33). ALDH is also highly expressed in human and murine hematopoietic stem and progenitor cells (20, 27, 28). ALDH1, a well-characterized member of the human ALDH family, has been found to be a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome (34). ALDH activity was also very useful for isolation of tumorigenic cells in some human colorectal xenografts (35).

In the present study, we found that ALDH and CD44 expression differed in two PCa cell lines. We found no $ALDH^{hi}$ expression in another PCa cell line, C42B. Consistent with the results of Patrawala *et al.* (13), PC-3 cells had very high expression of CD44, whereas it was undetectable in LNCaP cells We found that ALDHhi LNCaP cells, $ALDH^{hi}$ $CD44^{+}$ and $ALDH^{hi}$ $CD44^{-}$ PC-3 cells all demonstrated a higher colony-forming ability than $ALDH^{lo}$ cells, supporting the results of Hess *et al.* (20) that $ALDH^{hi}$ cells possess *in vitro* hematopoietic progenitor function. The role of CSCSs in multistage cancer progression, particular with respect to metastasis, has been investigated recently. Hermann *et al.* found that a distinct subpopulation of pancreatic cancer stem cells was identified that determines the metastatic phenotype of the individual tumor; meanwhile, depletion of the CSC pool for these migrating CSCs virtually abrogated the metastatic phenotype of pancreatic tumors without affecting their tumorigenic potential (21). CD44 has also been implicated in breast and PCa metastasis (7). Our data demonstrated that both

ALDH^{hi} and CD44^{+} cells are much more invasive than ALDH^{lo} CD44^{-} cells, suggesting the high metastatic ability of ALDH^{hi} CD44^{+} cells. Several groups have reported that CSC-like cells can be isolated from established tumor cell lines by culturing these cells in serum-free media with selected growth factors such as platelet-derived growth factor (PDGF), biotin-conjugated epidermal growth factor (bEGF) and epidermal growth factor (EGF) (15, 36). Miki *et al*. (26) found that human telomerase reverse transcriptase (hTERT)-immortalized cells with high CD133 expression exhibited 'prostaspheres' in non-adherent culture systems. The data presented here showed that ALDH^{hi} CD44^{+} cells retain a spheroid characteristic in a non-adherent system. Meanwhile, they grow very slowly in serum-free media, which is consistent with stem cell traits. Most interestingly, even in an adherent system with regular medium, ALDH^{hi} LNCaP cells formed typical spheroid colonies, indicating that ALDH activity might correlate with the sphere-forming ability of stem cell-like cells.

The experiment of tumorigenic potential in NOD/SCID mice demonstrated that ALDH^{hi} CD44^{+} cells had greater tumorigenic potential than did ALDH^{lo} CD44^{-} cells. However, ALDH^{lo} CD44^{-} cells did develop tumors, albeit with long latencies. A similar result was also reported by Patrawala *et al*. (13), who reported that CD44^{-} DU145 PCa cells developed tumors when injected into mice. These data demonstrate that CD44^{-} cells can establish tumors, which suggests that CD44 is not a requisite marker of CSCs in PCa cell lines. However, it is possible that this result was observed due to ALDH^{hi} or CD44^{+} cells that were present in the sorted cells due to inefficiency in flow sorting.

A requisite characteristic of stem cells is their ability to self renew. In terms of CSCs, this implies that they will form tumors at the same ratio of different cell populations as that observed in the original tumor they were derived from. Analysis of the dissociated tumor cells in the current study demonstrated that all permutations of cell types were able to form tumors that recapitulated the cell distribution of the original unsorted tumor. Thus, ALDH^{lo} and CD44^{-} cells both had self-renewal ability.

Whether cell lines can serve as *in vitro* models for CSCS study still remains controversial. There are several disadvantages in utilization of this *in vitro* model. Firstly, it cannot replicate exact *in vivo* conditions; secondly, during the long-term culture process, some cell property changes might take place such as gene alterations; thirdly, the *in vitro* cultured cells often lose their original differentiated function, and cannot stably maintain the exact properties of the original organ. Regardless of these disadvantages, the cell lines still produce heterogeneous and hierarchical subpopulations (37). To date, several CSCs studies using cell lines have been successfully performed (13, 15, 38). In cultured PCa cells and many other cancer cell lines, including those of the breast, colon, bladder, cervix, and ovary as well as glioma and melanoma cells, Patrawala *et al*. found a positive correlation between CD44 expression and tumor cell malignancy in most cases (13). Kondo *et al*. also utilized SP to study CSC-like cells in the C6 glioma cell line (15). Harper *et al*. proved that cell lines derived from head and neck squamous cell carcinoma contain cells with stem cell properties and that such cell lines may provide experimental systems relevant to the behavior of stem cells present in the tumors of origin and to their responses to therapy (38).

In summary, our current experimental data suggest that ALDH activity and CD44 do not represent markers of classical CSCs in PCa cell lines; however, they do correlate with increased tumorigenic and invasive potential, and, as such, may be important molecules to be explored further for their role in PCa progression.

Acknowledgements

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