Characterization and Clinical Relevance of ALDH\textsuperscript{bright} Populations in Prostate Cancer

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Abstract

**Purpose:** High aldehyde dehydrogenase (ALDH) has been suggested to selectively mark cells with high tumorigenic potential in established prostate cancer cell lines. However, the existence of cells with high ALDH activity (ALDH\textsuperscript{bright}) in primary prostate cancer specimens has not been shown so far. We investigated the presence, phenotype, and clinical significance of ALDH\textsuperscript{bright} populations in clinical prostate cancer specimens.

**Experimental Design:** We used ALDEFLUOR technology and fluorescence-activated cell-sorting (FACS) staining to identify and characterize ALDH\textsuperscript{bright} populations in cells freshly isolated from clinical prostate cancer specimens. Expression of genes encoding ALDH-specific isoforms was evaluated by quantitative real-time PCR in normal prostate, benign prostatic hyperplasia (BPH), and prostate cancer tissues. ALDH1A1-specific expression and prognostic significance were assessed by staining two tissue microarrays that included more than 500 samples of BPH, prostatic intraepithelial neoplasia (PIN), and multistage prostate cancer.

**Results:** ALDH\textsuperscript{bright} cells were detectable in freshly excised prostate cancer specimens ($n = 39$) and were mainly included within the EpCAM\textsuperscript{+} and Trop2\textsuperscript{+} cell populations. Although several ALDH isoforms were expressed to high extents in prostate cancer, only ALDH1A1 gene expression significantly correlated with ALDH activity ($P < 0.01$) and was increased in cancers with high Gleason scores ($P = 0.03$). Most importantly, ALDH1A1 protein was expressed significantly more frequently and at higher levels in advanced-stage than in low-stage prostate cancer and BPH. Notably, ALDH1A1 positivity was associated with poor survival ($P = 0.02$) in hormone-naïve patients.

**Conclusions:** Our data indicate that ALDH contributes to the identification of subsets of prostate cancer cells of potentially high clinical relevance.

Introduction

Despite the availability of several therapeutic options, prostate cancer remains a leading cause of cancer-related death in men (1). Prostate cancer is characterized by remarkable cellular heterogeneity and includes cells with different phenotypes, proliferative capacities, and differentiation states. However, the clinical significance and the prognostic relevance of specific cancer cell subpopulations are still unclear (2).

Aldehyde dehydrogenase (ALDH) enzyme is responsible for the oxidation of cellular aldehydes resulting in the production of retinoic acid (3). Notably, ALDH has been shown to be involved in stem cell protection and differentiation, and high levels of ALDH activity have been found in several stem cell populations (4, 5). Thus, high ALDH activity has been used to select and identify normal hematopoietic stem cells (4, 6) and tumor-initiating cells (TIC) in hematopoietic malignancies (7).

TICs, functionally defined as cells capable of initiating tumors in immunodeficient mice (8), have also been identified in a variety of human solid tumors (8–11). In a number of cancers of diverse histologic origin, TICs have been reported to exhibit high levels of ALDH activity (5, 12–14).

In the prostate, high ALDH activity has been shown to represent a functional marker for murine normal progenitor/stem cells (15). More recently, cells exhibiting high ALDH activity, referred to as "ALDH\textsuperscript{bright}" cells, have been...
Translational Relevance

High aldehyde dehydrogenase (ALDH) activity has been shown to be associated with tumorigenesis and proposed to represent a functional marker for tumor-initiating cells (TIC) in various tumor types, including prostate cancer. As previous studies were mainly based on the use of established cell lines, clinical relevance of ALDH bright populations has not been investigated in detail so far in prostate cancer. Here, using a large cohort of multistage prostate cancer specimens, we show that ALDH bright populations are present and heterogeneously distributed in prostate cancer tissues. Furthermore, we show that expression of the ALDH1A1-specific isoform, at both the gene and protein levels, is associated with advanced clinical stage and unfavorable prognosis in hormone-naive prostate cancer. Our findings highlight the potential importance of ALDH in prostate cancer pathogenesis and suggest that its clinical significance might be specific for patient subgroups. These results might help improve stratification and identification of high-risk patients with prostate cancer.

identified in human prostate cancer cell lines and expanded primary prostate cancer cultures (16). ALDH bright cells isolated from established prostate cancer cell lines were found to be associated with increased clonogenicity, invasiveness, as well as high tumorigenic and metastatic capacities (16, 17). However, culture conditions might modulate cell characteristics, potentially favoring selection of specific phenotypes, and prostate cancer cell lines may therefore be inadequate to reflect the biology of human prostate cancer (18–21).

To date, there is no evidence of the existence and functional characterization of ALDH bright populations in uncultured clinical prostate cancer specimens. In addition, it is still unclear whether such cells have clinical and prognostic relevance in prostate cancer.

In this study, we have identified, quantified, and characterized ALDH bright populations in cells isolated from freshly excised clinical prostate cancer specimens. Our results indicate that ALDH bright subsets are detectable to various extents in all clinical prostate cancer samples. Moreover, expression of several ALDH specific isoforms was increased in cancers as compared with benign samples.

Furthermore, we have assessed localization and clinical relevance of cells expressing ALDH1A1 isoform using 2 distinct tissue microarrays (TMA). Our results support an association between ALDH1A1 positivity and poor prognosis in prostate cancer.

Materials and Methods

Clinical specimens

A series of 38 patients with benign prostatic hyperplasia (BPH) and 71 patients with pT2a-pT3b stage prostate cancer referred for treatment at the Department of Urology of the University Hospital of Basel (Switzerland) from 2008 to 2012 was studied. Patients with BPH underwent conventional transurethral resection (TUR-P), whereas patients with prostate cancer underwent radical prostatectomy (RP). Written informed consent was obtained from all patients in accordance with the requirements of the local Ethical Committee (EKBB, Ref.Nr.EK: 176/07). Clinical and pathologic data of the patients with prostate cancer included in the present study are summarized in Supplementary Table S1.

Isolation of primary cells freshly derived from prostate cancer surgical specimens

Prostate specimens were examined for the presence of cancer tissues by experienced pathologists. Prostate cancer samples were chopped, washed, and digested in a mixture containing Dulbecco’s Modified Eagle’s Medium (DMEM), 5% knockout serum replacement (KO serum, Gibco), 1% Pen/Strep, and 200 IU/ml of type I collagenase (Worthington). After an overnight incubation at 37°C, digested tissues were washed and centrifuged as previously described to separate the epithelial and stromal fractions (22). Cell pellets enriched in the epithelial fraction were resuspended in PBS and passed through a 100 μm cell strainer to obtain single-cell suspensions. Cells were immediately used for fluorescence-activated cell-sorting (FACS) analysis to assess ALDH activity and surface markers expression (see below).

Quantification of gene expression by quantitative real-time PCR

A normal human prostate RNA pool was purchased from Clontech (Clontech Laboratories Inc.).

Total cellular RNA was extracted from tissues, and DNase treated by using Nucleospin RNA II (Macherey-Nagel). RNA was reverse-transcribed by using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT, Invitrogen). Quantitative real-time PCR (qRT-PCR) assays were conducted and specific gene expression was analyzed as previously described (23). TMPRSS2-ERG primers and probe sequences were derived from existing literature (24). Primers and probes for ALDH1A1, ALDH1A3, ALDH3A1, ALDH4A1, ALDH7A1, ALDH9A1, and ALDH18A1 specific isoforms were provided by Assays-on-Demand, Gene Expression Products (Applied Biosystems).

Identification and isolation of cells with high ALDH activity (ALDH bright cells)

ALDH activity was assessed by using the ALDEFLUOR Assay System (StemCell Technologies) according to the manufacturer’s recommendations. Single cells obtained from prostate cancer specimens were resuspended in ALDEFLUOR buffer and incubated with the ALDH substrate, biodipy-aminoacetaldehyde (BAAA). As negative control, an aliquot of the treated cells was also incubated with the ALDH inhibitor, diethylaminobenzaldehyde (DEAB). Following 35 to 40 minutes of incubation at 37°C, cells were washed and analyzed using a dual laser BD FACS Calibur (BD Biosciences). Dead cells were excluded on the basis of propidium
iodide (PI) incorporation. Results are presented as percentages of ALDHbright cells or as ratios of mean fluorescence intensities (MFI) as compared with control cells incubated with DEAB.

Phenotypic characterization of ALDHbright cells

Following treatment with ALDEFLUOR assay system, cells were resuspended in buffer and incubated with phycoerythrin (PE)-labeled anti-CD44 (BD Biosciences), allophycocyanin (APC)-labeled anti-EpCAM (BD Biosciences), and APC-labeled anti-Trop2 (R&D Systems) antibodies or isotype-matched immunoglobulins, at concentrations recommended by the manufacturers.

Tissue microarrays (TMAs)

The 2 prostate TMAs used in this study were constructed as previously described (25). The "progression TMA" contained single tissue cores from prostate cancers from all stages (25). The "castration-resistance TMA" has been recently described (26) and addresses the progression from hormone-naïve (HN) to lethal castration-resistant (CR) prostate cancer (27). Characteristics and number of samples included in the 2 TMAs are given in Table 1.

Immunohistochemistry

Immunohistochemical analyses were conducted according to standard indirect immunoperoxidase procedures as previously reported (25). Expression of ALDH1A1 in TMAs was assessed using a specific rabbit polyclonal antibody (ab51028, Abcam) at 1:200 (pretreatment: heat-mediated antigen retrieval at 100 °C). Other immunohistochemical procedures are described in detail in the Supplementary Information.

TMA analysis

Staining was visually scored and stratified into 4 groups: negative (absence of staining), weak (weak but distinct immunoreactivity), moderate (between weak and strong), and strong intensity (apparent even at low magnification: ×2.5 objective). As previously described, a histoscore (H-score) was calculated by multiplying the staining intensity (0, 1, 2, or 3) by the percentage of positive cells, leading to an H-score ranging from 0 to 300 (25). A score greater than 0 was considered positive. Stainings were independently evaluated by 2 experienced members of the team (L. Bubendorf and J.R. Gsponer) with excellent correlation between paired measurements.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism5.0 (GraphPad Software Inc.) and SPSS softwares (IBM). To assess the equality of means, parametric t test or nonparametric Mann–Whitney test were used. Correlation of specific expression between 2 groups was assessed using Pearson or Spearman correlation tests. Fisher and χ² tests were used to compare ALDH1A1 positivity frequency in different groups of patients. Survival curves were constructed according to Kaplan–Meier and compared using log-rank (Mantel–Cox) tests. P < 0.05 was considered statistically significant.

Results

Epithelial cells freshly isolated from prostate cancer clinical specimens contain a heterogeneous ALDHbright population

Presence of ALDHbright cells was investigated in cells freshly isolated from 39 prostate cancer specimens directly retrieved following surgery. Representative cytograms are shown in Fig. 1A. As shown in Fig. 1B, ALDHbright populations could be detected in all cell preparations obtained from clinical prostate cancer samples. Percentages of ALDHbright cells were highly variable and differed from patient to patient (average ±SE = 1.56% ± 0.24% bright cells; MFI ratio = 257.2 ± 40.87; n = 39). When gated on EpCAMbright cells to identify the bulk of the epithelial population, percentages of ALDHbright cells were significantly higher and exceeded 15% of EpCAMbright cells in several specimens (average ±SE = 9.06% ± 0.97% bright cells; MFI ratio = 292.3 ± 32.99; n = 31). As shown in Fig. 1C, a trend toward a higher percentage of ALDHbright cells in high (G8-9) as compared with medium (G7) Gleason score was evident (P = 0.09).

Table 1. Immunohistochemical analysis of ALDH1A1 expression on TMAs

<table>
<thead>
<tr>
<th></th>
<th>Total interpretable</th>
<th>Negative n (%)</th>
<th>Positive n (%)</th>
<th>Average score</th>
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<tr>
<td></td>
<td>n (% )</td>
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<tr>
<td>Progression TMA</td>
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<tr>
<td>BPH</td>
<td>54 (100%)</td>
<td>52 (96%)</td>
<td>2 (4%)</td>
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<td>PIN</td>
<td>38 (100%)</td>
<td>35 (92%)</td>
<td>3 (8%)</td>
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<tr>
<td>T1a/b</td>
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<td>47 (96%)</td>
<td>2 (4%)</td>
<td>9.0</td>
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<tr>
<td>RP (pT2-pT3b)</td>
<td>69 (100%)</td>
<td>57 (83%)</td>
<td>12 (17%)</td>
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<tr>
<td>CR</td>
<td>80 (100%)</td>
<td>65 (82%)</td>
<td>15 (18%)</td>
<td>18.3</td>
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<tr>
<td>Castration resistance TMA</td>
<td></td>
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<tr>
<td>BPH</td>
<td>11 (100%)</td>
<td>10 (91%)</td>
<td>1 (9%)</td>
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<td>100 (100%)</td>
<td>60 (60%)</td>
<td>40 (40%)</td>
<td>40.5</td>
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<tr>
<td>CR</td>
<td>107 (100%)</td>
<td>67 (63%)</td>
<td>40 (37%)</td>
<td>33.7</td>
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</table>
Phenotypic characterization of ALDH<sup>bright</sup> cells in prostate cancer clinical specimens

The epithelial cell–specific marker EpCAM has been shown to be highly expressed in a majority of carcinomas including prostate cancer (28) and has also been proposed as TIC marker in a variety of solid tumors (29). Interestingly, the majority of the ALDH<sup>bright</sup> population was also positive for EpCAM (average ± SE = 75.47% ± 4.06% of EpCAM<sup>+</sup> cells within the ALDH<sup>bright</sup> subset; Fig. 2A and B). Expression of Trop2, previously shown to be expressed by epithelial prostate cells (30) and to be overexpressed in human prostate cancer, was also tested (31, 32). Likewise, a high proportion of ALDH<sup>bright</sup> cells was also positive for Trop2 (average ± SE = 81.41% ± 4.94% Trop2<sup>+</sup> cells within the

Figure 1. Identification of ALDH<sup>bright</sup> populations in cells freshly isolated from prostate cancer (PCa) surgical specimens. A, flow cytometric analysis of cells from 3 representative patients with prostate cancer (#1–3). Single-cell suspensions were obtained after digestion of prostate cancer tissues. ALDH activity was tested on live cells (left) using the ALDEFLUOR technology. Cells were also tested for EpCam-specific expression to identify ALDH<sup>bright</sup> cells within the EpCam<sup>+</sup> population (right). B, cell suspensions freshly derived from 39 prostate cancer specimens were assessed for ALDH activity (“All”). For 31 specimens, ALDH activity was also tested in the EpCam<sup>+</sup> population [“EpC(<sup>+</sup>)”]. Left, percentages of ALDH<sup>bright</sup> cells. Right, MFI ratios to the DEAB control. C, percentage of ALDH<sup>bright</sup> cells in the EpCam<sup>+</sup> population in patients with Gleason 7 (G7) as compared with patients with high (G8-9) Gleason score. Four patients with Gleason 5–6 were not included in the analysis.
ALDH bright subset; Fig. 2B). To gain additional insights into the phenotype of ALDH bright cells in prostate cancer, ALDE-FLUOR-treated prostate cancer cells were co-stained with antibodies recognizing EpCAM and CD44 (n = 4). In all cases, ALDH bright phenotype was heterogeneous, as illustrated by 2 representative staining profiles in Fig. 2C. Two populations of EpCAM high cells were present within the ALDH bright population, either positive or negative for CD44. In one particular prostate cancer case, an EpCAM/C0 CD44 high subset was detected in the ALDH bright population.

ALDH-specific isoforms are highly expressed in prostate cancer clinical specimens

We next investigated the expression of selected ALDH-specific isoform genes. High expression of all tested ALDH isoforms was found in all prostate cancer tissues (Fig. 3A, left). Expression of these genes was then comparatively evaluated in prostate cancer, BPH, and normal prostate. ALDH3A1 was the only isoform displaying a significantly lower gene expression in prostate cancer than in BPH (Fig. 3A). In contrast, a trend toward higher expression of ALDH1A1, ALDH4A1, and ALDH9A1 was observed in prostate cancer as compared with BPH and normal tissues (Supplementary Table S2 and Fig. 3A). The ALDH1A3, ALDH7A1, and ALDH18A1 isoforms were expressed at significantly higher levels in prostate cancer than in BPH specimens and normal tissues (Supplementary Table S2 and Fig. 3A). The same trends were observed when selected prostate cancer samples found to be positive for the tumor-specific TMPRSS2-ERG gene fusion (33) were used (Supplementary Table S2).

Expression of ALDH isoform genes was then comparatively analyzed in prostate cancer with different Gleason scores. Interestingly, expression of ALDH1A1 was upregulated in high-grade (G8-9) as compared with low-grade (G5-6) cancers (p = 0.03; Fig. 3B). The other isoforms did not show any differential gene expression in high-grade as opposed to low-grade cancers (data not shown).
ALDEFLUOR reagent is generally thought to act as a substrate for the ALDH1A1 isoform (34). However, in breast cancer, ALDEFLUOR-dependent ALDH activity has been attributed to the ALDH1A3 isoform (35). In prostate cancer, ALDH1A1 was the only isoform, whose gene expression correlated with levels of ALDH activity as detected in the same tissue specimens (Fig. 3C). These data indicate that ALDH1A1 is the main isoform contributing to measurable ALDH activity in primary prostate cancer.

**Expression and localization of ALDH1A1 protein in prostate tissues**

ALDH1A1 gene expression appears to be higher in tumors of higher grade and correlates with enzymatic activity. To gain insights into its potential clinical relevance, we investigated the expression of ALDH1A1 protein in a series of BPH, prostatic intraepithelial neoplasia (PIN), and multi-stage prostate cancer specimens included in 2 TMAs. Numbers and characteristics of samples represented in the TMAs are given in Table 1.

![Figure 3](image)

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To investigate ALDH1A1 protein expression and localization, we first used a prostate "progression TMA" comprising 290 evaluable samples including BPH, PIN, early-stage prostate cancer (T1a/b), radical prostatectomy (RP) specimens, and castration-resistant (CR) prostate cancer samples. In BPH, ALDH1A1 protein was detectable in 3 of 65 specimens (4.6%, Table 1). In the few positive BPH cases,
ALDH1A1 protein expression was clearly restricted to a few cells in the basal layer of the epithelium (Fig. 4A–C). In contrast, ALDH7A1 protein in BPH was uniformly expressed in both basal and luminal cells (Supplementary Fig. S1).

To strengthen these data, additional staining of larger sections from BPH specimens was analyzed. This analysis confirmed that in positive BPH samples, ALDH1A1 protein expression is usually limited to a few epithelial basal cells (Supplementary Fig. S2B). In addition, ALDH1A1 protein was also detectable in nonepithelial cells, such as histiocytes and peripheral nerve cells (Supplementary Fig. S2C and S2D).

In PIN and low-stage T1a/b prostate cancer samples, ALDH1A1 positivity was detectable in 3 of 38 (8%) and 2 of 47 samples (4%), respectively (Table 1). For the few positive PIN samples, ALDH1A1 protein expression was specifically localized in PIN premalignant cells but not in benign glands (Fig. 4D and E).

Consistent with the PIN data, ALDH1A1 positivity was specifically detectable in cancerous cells of RP samples (Fig. 4F and G). In CR samples, ALDH1A1 expression showed large variations, ranging from fully negative (Fig. 4H), to focally or diffusely positive tumors (Fig. 4I). Interestingly, ALDH1A1(+) cells were also present in the peri-epithelial stroma (Fig. 4D).

Importantly, ALDH1A1 protein expression was more frequent in RP (12 of 69, 17%) and CR (15 of 80, 18%) than in BPH (P = 0.02 and P = 0.01, respectively) or low-stage samples (P = 0.04 and P = 0.02, respectively; Fig. 4A and Table 1). Using ALDH1A1 scoring, the same trends were observed by comparing RP and CR with BPH (P = 0.01 and P = 0.003, respectively; Fig. 4A). However, we did not find significant differences in percentages of ALDH1A1(+) tissues or global score among samples with different Gleason scores or Ki67 levels (data not shown).

**ALDH1A1 expression is a predictor of poor prognosis in hormone-naive patients**

To evaluate the prognostic relevance of ALDH1A1 in prostate cancer, ALDH1A1 expression was examined in the *castration resistance TMA* which contained hormone-naive (HN) and CR samples with complete follow-up data. Consistent with the results from the *progression TMA,* ALDH1A1 protein was detectable with significantly higher frequency in HN (40 of 100, 40%) and CR (40 of 107, 37%) prostate cancer than in BPH (Table 1). Score data were also comparably different (HN vs. BPH: P = 0.03; CR vs. BPH: P = 0.04).

In the HN patient set, the median survival (MS) was 34 months for patients with ALDH1A1-positive tumors and 56 months for patients with ALDH1A1-negative tumors (P = 0.02; Fig. 5A).

In the CR group, however, MS for patients with ALDH1A1-positive or -negative tumors was similar (13 and 10 months, respectively), and no correlation was observed between ALDH1A1 expression and patients overall survival (P = 0.89; Fig. 5B).

**Discussion**

ALDHs are detoxification enzymes that catalyze the oxidation of various aldehydes. Their dysfunction is involved in several types of disease including cancer (3). High ALDH activity was shown to characterize hematopoietic stem cells and to select for TICs in various types of tumors (5, 12–14), supporting a link between ALDH expression and carcinogenesis.

High ALDH activity was also proposed to select for highly tumorigenic cells in prostate cancer cell lines (16, 17, 36). To date, however, there is no evidence for the existence of ALDHbright populations in primary prostate cancer. In the present work, we therefore investigated presence, prevalence, characteristics, and clinical relevance of ALDHbright populations in primary prostate cancer.

First, we determined whether ALDHbright cell populations were present in cells freshly isolated from clinical prostate cancer specimens collected after surgery. We successfully identified and quantified ALDHbright populations among cell suspensions freshly isolated from 39 clinical prostate cancer specimens. To our knowledge, this is the first study reporting the identification of cells with high functional ALDH activity in uncultured prostate cancer specimens. Overall, ALDHbright cells could be detected with variable and relatively high frequency in most prostate cancer samples with a trend toward higher percentages in cancers with higher histologic grade.

We found that high ALDH activity, in prostate cancer, mainly localized to cells highly positive for the epithelial markers EpCAM and Trop2, suggesting an epithelial origin for these cells. Notably, both these markers have been reported to be highly expressed in prostate cancer (28, 31).

We also tested whether cells with high ALDH activity expressed CD44, a mostly basal cell–specific marker. Interestingly, both CD44-positive and -negative phenotypes were observed within the ALDHbright population. Our preliminary analysis paves the way toward a thorough phenotypic characterization of ALDHbright populations in primary prostate cancer.

The ALDEFLUOR assay is the sole accurate method to assess functional ALDH activity but is unsuitable to investigate the clinical and prognostic relevance of ALDHbright populations in large cohorts of archived specimens (34).

To date, 19 ALDH isoforms with different substrate specificities have been identified in the human genome (3). Among these, ALDH1A1 is generally considered to be the main isoform contributing to ALDH activity, as measured by ALDEFLUOR (34). However, evidence for the contribution of other isoforms in solid tumors has recently been obtained. In fact, ALDH1A3 rather than ALDH1A1 has been identified as the main isoform involved in ALDEFLUOR-measured ALDH activity and a strong predictor of metastasis in breast cancer (35). Moreover, ALDH7A1 has recently been shown to contribute to ALDH activity in a
Figure 4. Expression of ALDH1A1 protein in benign and cancerous prostate tissues. A, ALDH1A1 is expressed more frequently (left) and at higher levels (right) in RP and CR prostate cancer (PCa) than in BPH and low-stage prostate cancer (T1a/b). *, P < 0.05; **, P < 0.01. B–I, representative pictures of ALDH1A1 expression in a panel of prostate tissues. B, negative BPH glands. C, BPH glands exhibiting a few positive basal cells. D, interstitial stromal cells showing some degree of positivity. E, positive PIN lesions. F and G, positive cancer glands (brown arrow) and negative benign glands (right arrow). H, negative CR cancer. I, CR with diffuse positivity. Brown arrows indicate positive areas enlarged and shown in inserts. Magnification: B, F: ×200, C, D, E, G, H, I: ×400.
ALDH Activity in Prostate Cancer

PC3-derived cell line and to be involved in bone metastasis in prostate cancer (37). Thus, it appears that the nature of the isoforms contributing to high ALDH activity, as detected by the ALDEFLUOR assay, is tissue- and cell-specific (34). To identify prostate cancer relevant isoforms, we have tested the expression of seven ALDH isoforms, previously shown to be implicated in ALDH activity, as detected by ALDEFLUOR, and in cancer initiation (reviewed in ref. 34). Except for ALDH3A1, we observed a trend toward higher expression of ALDH isoforms in prostate cancer than in BPH and normal samples. In particular, ALDH1A3, ALDH7A1, and ALDH18A1 were expressed to significantly higher extents in prostate cancer than BPH. ALDH1A1 gene was not expressed to significantly higher extents in prostate cancer than BPH (P = 0.12) but we observed a significantly higher gene expression in high-grade than in low-grade prostate cancer. Importantly, ALDH1A1 was the sole isoform whose gene expression was correlated with high ALDH activity detected in the same specimens, suggesting its major contribution to functional ALDH activity in prostate cancer.

These results led us to investigate expression of ALDH1A1 at the protein level, in a large cohort of benign and cancerous samples. In the 2 TMAs analyzed, we found a minor (<5%) proportion of BPH samples with ALDH1A1 positivity restricted to a few cells in the basal layer of the epithelium, in agreement with a recent study (38). This was confirmed by staining of large tissue sections.

In contrast, ALDH1A1 expression was specifically detected in luminal cancer cells and showed broad variations, ranging from full negativity to focal, diffuse, or strong positivity. These expression patterns highlight the patient-to-patient heterogeneity common in prostate cancer. ALDH1A1 expression was higher and more frequently found in RP and CR than in early-stage prostate cancer. We also observed intratumor heterogeneity with concomitant presence of negative and positive glands within the same samples. In these cases, ALDH1A1 expression was convincingly restricted to cancerous glands. Interestingly, ALDH1A1 positivity was also found in a subgroup of PIN (8%). While these samples usually contain a mixture of benign and PIN glands, ALDH1A1 expression was restricted to PIN lesions. Because PIN is a precursor for prostate cancer (39), these findings may have implications for early identification of patients at high risk of developing prostate cancer.

Next, we assessed the prognostic relevance of ALDH1A1 expression using a “castration resistance” TMA. In the hormone-naïve cohort, we found a significant correlation between ALDH1A1 positivity and poor patient outcome. These results are consistent with a study by Li and colleagues, who evaluated a smaller and less diversified patient cohort (38). In contrast, in patients with castration-resistant disease, no significant correlation was found between ALDH1A1 positivity and clinical outcome. These data may suggest a prognostic relevance of ALDH activity limited to early phases of prostate cancer.

ALDH1A1+ cells have been proposed to be responsible for tumor re-initiation after castration (40, 41). Our data, however, indicate that these cells are not likely associated with prognostic significance in CR prostate cancer, suggesting that ALDH1A1-negative cells might acquire a predominant clinical relevance following cancer progression to androgen independence.

High ALDH activity has previously been shown to identify TICs in solid cancers (5, 12, 13) and established prostate cancer cell lines (16). On the basis of this background, it would be tempting to speculate that ALDH$^{bright}$ primary prostate cancer cells might possess tumor-initiating capacity. However, a formal demonstration of the TIC nature of these ALDH$^{bright}$ primary prostate cancer cells would require direct testing of their tumorigenicity in vivo. These studies are hampered by a lack of appropriate experimental models and the difficulties inherent in the generation of primary cultures and xenografts derived from prostate cancer primary cells (19, 20). Recent successful attempts are based on the implantation of tumor fragments under the renal capsules of immunodeficient mice in the presence of androgen-releasing pellets (42). Yet generation of cell suspensions from clinical samples and cell sorting are known to affect viability of prostate cancer cells (42, 43), therefore preventing successful tumor transplantation (reviewed in ref. 19). To the best of our knowledge, tumor transplantation of cells derived from primary human prostate cancer, as selected by the expression of putative TIC markers, has never been reported so far (44). This prevented us from testing the tumorigenicity of ALDH$^{bright}$ primary prostate cancer cells.
Alternatively, considering its expression in rare benign basal cells and in some untransformed interstitial cells, in addition to tumor cells, ALDH1A1 could represent a marker associated with undefined cell differentiation stages, irrespective of a putative role in cancer initiation. Nevertheless, our data clearly document a prognostic relevance of ALDH1A1 expression in untreated prostate cancer. This might have important implications for the identification of patients at high risk for progression to castration-resistant disease.

In summary, we provide novel evidence of cells with high ALDH functional activity in primary prostate cancer. Detection at functional, mRNA, and protein levels suggests that ALDH might be involved in prostate cancer outgrowth and progression. However, this involvement might be specific for subgroups of patients as suggested by the high variability observed between prostate cancer specimens.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: C. Le Magnen, C. Mengus, G.N. Thalmann, M.G. Cecchini, M. Germann, G.C. Spagnoli

Development of methodology: C. Le Magnen, C. Mengus, G.C. Spagnoli

References

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Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, computational analysis): C. Le Magnen, C. A. Rentsch, J. R. Coponter, G. N. Thalmann, G. C. Spagnoli

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