Defining a Population of Stem-like Human Prostate Cancer Cells That Can Generate and Propagate Castration-Resistant Prostate Cancer

Xin Chen1,2,3, Qiuhui Li1,3, Xin Liu1, Can Liu1, Ruiyang Liu1,3, Kiera Rycal1,3, Dingxiao Zhang1,3, Bigang Liu1, Collene Jeter1, Tammy Calhoun-Davis1, Kevin Lin1, Yue Lu1, Hsueh-Ping Chao1, Jianjun Shen1, and Dean G. Tang1,3,4,5

Abstract

Purpose: We have shown that the phenotypically undifferentiated (PSA−/lo) prostate cancer cell population harbors long-term self-renewing cancer stem cells (CSC) that resist castration, and a subset of the cells within the PSA−/lo population bearing the ALDH−CD44+/α2B1+/CD133− phenotype (Triple Marker+/TM+) is capable of robustly initiating xenograft tumors in castrated mice. The goal of the current project is to further characterize the biologic properties of TM+ prostate cancer cell population, particularly in the context of initiating and propagating castration-resistant prostate cancer (CRPC).

Experimental Design: The in vitro CSC activities were measured by limiting-dilution serial cell tumor transplantation assays in both androgen-dependent and androgen-independent prostate cancer xenograft models. In vitro clonal, clonogenic, and sphere-formation assays were conducted in cells purified from xenograft and patient tumors. qPCR, Western blot, lentiviral-mediated gene knockdown, and human microRNA arrays were performed for mechanistic studies.

Results: By focusing on the LAPC9 model, we show that the TM+ cells are CSCs with both tumor-initiating and tumor-propagating abilities for CRPC. Moreover, primary patient samples have TM+ cells, which possess CSC activities in ‘castrated’ culture conditions. Mechanistically, we find that (i) the phenotypic markers are causally involved in CRPC development; (ii) the TM+ cells preferentially express castration resistance and stem cell–associated molecules that regulate their CSC characteristics; and (iii) the TM+ cells possess distinct microRNA expression profiles and mir-499-5p functions as an oncomir.

Conclusions: Our results define the TM+ prostate cancer cells as a population of preexistent stem-like cancer cells that can both mediate and propagate CRPC and highlight the TM+ cell population as a therapeutic target. Clin Cancer Res; 1–12. ©2016 AACR.

Introduction

Human tumors are heterogeneous, containing many phenotypically and functionally distinct cancer cells, and tumor cell heterogeneity can arise as a result of genetic diversity and/or epigenetic maturation of stem cell–like cancer cells or cancer stem cells (CSC; refs. 1, 2). CSCs in many human solid tumors have been implicated in tumor initiation, progression, metastasis, and therapy resistance (3, 4). Like other human cancers, prostate cancer is a heterogeneous malignancy containing phenotypically differentiated cancer cells and immature cancer cells with stem cell properties, i.e., prostate CSCs (PCSC, refs. 4–19).

Prostate cancer is one of the most common malignancies affecting American males, with an estimated 220,800 new cases and 27,540 cancer-associated deaths in 2015 (20). Localized prostate cancer at an early stage can be treated by radical prostatectomy with a good prognosis. Advanced prostate cancer patients are mostly treated by androgen-deprivation therapy (ADT), which fails eventually leading to the development of incurable and lethal castration-resistant prostate cancer (CRPC). Although many molecular mechanisms have been proposed to explain CRPC, the cellular origin for CRPC remains largely unknown (4).

In the field, the cell-of-origin of primary prostate cancer, i.e., whether human prostate cancer is derived from basal, luminal, or intermediate (progenitor) cells, has been an area of intensive studies. In mouse models, several groups have shown that both basal and luminal murine prostatic epithelial cells can function as the targets of tumorigenic transformation (21). In the human prostate, the basally localized stem cell–enriched population, i.e., CD49f+/Trop2−, can be transformed by Akt, ERG, and androgen receptor (AR) and can function as the cell-of-origin for human prostate cancer (22). Whether this cell population can serve as the cell-of-origin for CRPC is presently unknown. Recent evidence

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Authors: Qiuhui Li or Dean G. Tang, Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, New York. 1Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, Texas. 2Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. 3Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, New York. 4Cancer Stem Cell Institute, Research Center for Translational Medicine, East Hospital, Tongji University School of Medicine, Shanghai, China. 5Centers for Cancer Epigenetics, Stem Cell and Developmental Biology, RNA Interference and Noncoding RNAs, and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Houston, Texas.

Published OnlineFirst April 8, 2016; DOI: 10.1158/1078-0432.CCR-15-2956

©2016 American Association for Cancer Research.
Translational Relevance
Most advanced prostate cancer patients treated with androgen-deprivation therapy develop castration-resistant prostate cancer (CRPC). Although many molecular mechanisms have been proposed to explain CRPC, the cellular origin for CRPC remains mostly unclear. Systematic studies shown here demonstrate that the TM⁺ (i.e., ALDH⁺CD44⁺α2β1⁺) subpopulation of the prostate cancer cells preexist in untreated AD xenograft and patient tumors but become greatly enriched during castration in vitro and in vivo. Importantly, the TM⁺ cells can function not only as CRPC-initiating but also as CRPC-propagating cells, highlighting this population of the cells as a novel therapeutic target in treating prostate cancer. The availability of large numbers of TM⁺ cells in certain xenografts (e.g., LAPC9) should allow high-throughput screening to identify novel drugs targeting this population, which may eventually impact the clinical treatment of some patients with CRPC.

Materials and Methods

Cells, animals, and reagents
PC3, DU145, PPC-1, 22Rv1, and LNCaP cells were obtained from ATCC and cultured in RPMI-1640 plus 7% heat-inactivated fetal bovine serum (FBS), 10,000 µg/mL streptomycin, and 10,000 U/mL penicillin (PS; HyClone). These cell lines were regularly authenticated by our institutional CCSG Cell Line Characterization Core using short tandem repeat (STR) analysis and checked to be free of mycoplasma contamination using the Agilent MycoSensor QPCR Assay Kit (cat. #302107) every 8 weeks. All cell lines used were passaged fewer than 6 months after receipt. Antibodies used in this study are summarized in Supplementary Table S1. Immunodeficient mice (NOD/SCID and NOD/SCID-IL2Rγ⁻/⁻, i.e., NSG) were purchased from the Jackson Laboratory, and breeding colonies were maintained in our animal facility core under standard conditions. All animal-relevant studies in the current project have been approved by the MD Anderson Cancer Center IACUC (Institutional Animal Care and Use Committee; AClIF#00000923-RN00).

Limiting-dilution tumor regeneration assays (LDA) and serial tumor transplantations in immunodeficient mice
Basic procedures were previously described (16, 19). For LDA, FACS purified prostate cancer cells were mixed with Matrigel and injected subcutaneously (s.c.) at increasing numbers in NOD/SCID mice. For serial tumor transplantation assays, tumor cells of a specific phenotype were sorted from the first-generation (1⁰) tumors of the same phenotype for 2⁰ tumor transplantations. Sequential tumor transplantations were carried out using similar strategies. For tumor studies in castrated mice, male NOD/SCID or NOD/SCID-IL2Rγ⁻/⁻ (NSG) mice (8–10 weeks) were surgically castrated 1 to 2 weeks prior to tumor cell injections. At the endpoint, tumors were harvested and various parameters were recorded, including tumor incidence, weight, latency, and images. Tumor-initiating frequency (TIF) was calculated using the Limdil function of the Statmod package (http://bioinf.wehi.edu.au/software/elda/).

Gene knockdown with shRNA-encoding lentiviral vectors
Basic procedures have been previously described (16, 19). Briefly, we produced lentivirus in 293T packaging cells (Clontech), which was then tiered using GFP positivity in HT1080 cells. Prostate cancer cells were infected at a multiplicity of infection (MOI) of 10 to 20 for 48 to 72 hours at 37°C. All GIPZ-shRNA-encoding lentiviral vectors used in the current study were purchased from GE Dharmacon (Supplementary Materials and Methods). The knockdown effect on the target molecules was assessed by qRT-PCR.

Statistical analyses
We used an unpaired two-tailed Student t test to compare significance in cell numbers, percentages of CD44⁺ and/or α2⁺ cells, cloning and sphere-forming efficiencies, tumor weights, knockdown efficiency, mRNA levels of multiple genes and other related parameters. We used a χ² test to compare tumor incidence. All results were presented as mean ± SD with a P < 0.05 considered statistically significant.

See also Supplementary Materials and Methods.

Results

The TM⁺ (ALDH⁺CD44⁺α2β1⁺) prostate cancer cell population is enriched in experimental CRPC models
In our earlier cDNA microarray analysis, we compared gene expression profiles between PSA⁻/⁻ versus PSA⁺ LAPC9 prostate...
cancer cells and found that PSA−/lo prostate cancer cells overexpressed several dozens of stem cell–related genes, including CD44, integrin α2, and ALDH1A1 (16). ALDHhiCD44þα2β1 or TMþ LAPC9 cells regenerated much larger tumors when implanted in castrated mice than the corresponding ALDHloCD44/C0α2β1/C0 or TM/C0 cells (16), suggesting that TMþ prostate cancer cells may play an important role in CRPC development.

To directly test this suggestion, we established serially passaged androgen-independent (AI, i.e., castration-resistant) xenograft models, including LAPC9, LAPC4, LNCaP, and HPCa101 (25) from their respective androgen-dependent (AD) parental tumors (Fig. 1A). As illustrated in Fig. 1B, both LAPC9 and LAPC4 AI tumors showed a prominent upregulation of N-Cadherin, a molecule known to be involved in CRPC (24). In contrast, E-Cadherin levels did not significantly change in AI tumors in comparison to AD tumors (Fig. 1B). Interestingly, the LAPC4 AI tumors showed increased AR protein, whereas the LAPC9 AI tumors gradually lost AR, similar to earlier reports by others (24, 26). However, both AI tumor models showed decreased amounts of PSA (Fig. 1B), consistent with our earlier observations that castration resistance is associated with decreasing tumor cell PSA levels and increasing PSA−/lo PCSCs (16, 19). Together, these results indicate that we have successfully established experimental CRPC models.

Quantitative RT-PCR (qRT-PCR) analysis revealed that the LAPC9 AI tumors expressed significantly higher levels of integrin α2, ALDH1A1, and ALDH7A1 mRNAs than AD tumors (Fig. 1C). A trend of increased CD44 mRNA in LAPC9 AI tumors was also observed (Fig. 1C). Importantly, the CD44 and integrin α2 mRNAs levels in TMþ LAPC9 AI tumors were significantly higher than those in the corresponding TM− cells (Fig. 1D). FACS analysis, using the gating strategies we developed, showed that the percentage of TMþ cells dramatically increased in serially passaged LAPC9 AI tumors (Supplementary Fig. S1).

IHC and IF staining in formalin-fixed paraffin-embedded (FFPE) samples confirmed that CD44þ cells were highly enriched in LAPC9 AI tumors compared with AD tumor (Fig. 1E and F; and Figure 1. TMþ cells in AD and AI prostate cancer models. A, strategies in establishing AD and AI prostate cancer lines. B, Western blot analysis of the molecules indicated in AD and AI LAPC9 and LAPC4 tumors. Du145 and LNCap cells were used as controls. C, qRT-PCR analysis of mRNA levels for CD44, α2, ALDH1A1, and ALDH7A1 in LAPC9 AD and AI tumors. The relative transcript abundance was normalized to GAPDH levels. Error bars, mean ± SD. *, P < 0.05. D, qRT-PCR analysis of mRNA levels of CD44, α2 in sorted TMþ and isogenic TM− purified from 6Hi and 15L APC9 AI tumors. Bars, mean ± SD. *, P < 0.05. E–H, representative IF images of CD44 (E) and integrin α2 (G) staining in LAPC9 AD and serially passaged LAPC9 AI (e.g., 7AI and 13AI) tumors. Percentages of CD44þ (F) and α2þ (H) cells were characterized. Bars, mean ± SD. **, P < 0.01. I, TMþ prostate cancer cells were increased in LNCaP and HPCa101 AI tumors. K, TMþ prostate cancer cells were abundant in long-term cultured AI prostate cancer lines.

Figure 1.
Recent studies have linked aldehyde dehydrogenase (ALDH) activity in PCSCs to prostate cancer development (10, 11, 19, 27, 28) that might be conferred by several isoforms, including ALDH1A1 (10, 27, 28) and ALDH7A1 (11). However, whether these ALDH isoforms are expressed in CRPC samples is unclear. We found that the cells with high ALDH activity were increased in LAPC9 AI tumors (Supplementary Fig. S1, e), and the abundance of ALDH1A1\(^+\) and ALDH7A1\(^+\) prostate cancer cells was also higher in AI versus AD tumors (Supplementary Fig. S2B and S2C). In addition, integrin \(\alpha_2\)\(^+\) cells were increased in both LAPC9 (Fig. 1G and H) and LAPC4 (Supplementary Fig. S2D) AI tumor models.

We used FACS analysis to further investigate TM\(^+\) cells in several different models. In the LAPC9 model, \(\sim 1.7\%\) TM\(^+\) cells were present in AD tumors, but this percentage gradually increased with increasing cycles of castration to nearly 40\% at 6\(^{\text{th}}\) generation (Fig. 1I). The TM\(^+\) cells were rare in AD LNCaP and HPCa101 tumors, but their abundance still increased in the corresponding AI tumors (Fig. 1I). Finally, several commonly used AI prostate cancer cell lines all contained significant percentages of TM\(^+\) cells (Fig. 1K). Altogether, these results suggest that the TM\(^+\) prostate cancer cells are enriched in experimental CRPC models and are abundantly present in AI prostate cancer cell lines.

TM\(^+\) cells in AD tumors possess long-term tumor-propagating abilities in hormonally intact male mice

The 'gold standard' to measure CSC activity is to determine if a candidate CSC population has the capacity to generate serially xenotransplantable tumors in immunodeficient mice at increasing cell doses (an assay termed limiting-dilution tumor regeneration assay or LDA), and if the regenerated tumors phenocopy, at least partially, parental tumor histopathologically (4, 29). Therefore, LDA and serial transplantation assays are combined in our study to characterize the CSC properties of the TM\(^+\) related prostate cancer cell populations.

Because TM\(^+\) cells preexist in AD tumors (Fig. 1I–J; Supplementary Fig. S1A), we first determined their tumor-initiating and tumor-propagating activities in androgen-proficient hosts, using strategies similar to those we previously used to characterize the PSA\(^-\) cells (16). Thus, TM\(^+\) and several other cell populations, including ALDH\(^+\)CD44\(^-\)\(\alpha_2\)\(^+\)\(\beta_1\)\(^-\), ALDH\(^+\)CD44\(^-\)\(\alpha_2\)\(^+\)\(\beta_1\)\(^-\), and TM\(^-\), were purified from LAPC9 AD tumors and s.c. injected in hormonally intact NOD/SCID male mice at increasing doses (from 10 to 10,000 cells; Table 1). Surprisingly, at 1\(^\text{st}\), although TM\(^+\) cells exhibited a trend of higher tumorigenicity than corresponding TM\(^-\) cells (TIF 1/1312 vs. 1/2972; \(P = 0.0647\)), two intermediate cell populations, ALDH\(^+\)CD44\(^-\)\(\alpha_2\)\(^-\)\(\beta_1\)\(^-\) (TIF 1/315)

Table 1. TIF of subsets of LAPC9 cells in intact and castrated male NOD/SCID mice

<table>
<thead>
<tr>
<th>Cell dose</th>
<th>TIF (95% interval)</th>
<th>(P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 \times 10^4</td>
<td>10^4</td>
<td>10^3</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+)(\beta_1)(^+)</td>
<td>1 (^{\text{st}})</td>
<td>3/4</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{nd}})</td>
<td>1 (^{\text{st}})</td>
<td>1/2</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>3/4</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>2/4</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>3/4</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>2 (^{\text{nd}})</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>3/4</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>2 (^{\text{nd}})</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>3 (^{\text{rd}})</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>5 (^{\text{rd}})</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>8/8</td>
</tr>
<tr>
<td>ALDH(^+)CD44(^-)(\alpha_2)(^+) (^{\text{rd}})</td>
<td>1 (^{\text{st}})</td>
<td>0/4</td>
</tr>
</tbody>
</table>

\(^a\)TIF and statistical differences (\(P\) values) were determined using the Lilliford function of the Statmod package (http://bioinf.wehi.edu.au/software/elda/).

\(^b\)For these experiments, triple marker-positive and other marker-profile LAPC9 cells were purified from the AD xenograft tumors maintained in intact male NOD/SCID mice and injected s.c. in Matrigel at the indicated cell doses. Tumors were harvested 75 to 149 days after implantation depending on the animal well-being and IACUC regulations. Then, tumor cells of a specific phenotype were purified from the 3rd tumors of the same phenotype (e.g., purifying three marker-positive cells from a triple marker-positive tumor) for 3rd transplantsations. Likewise, tumor cells of a specific phenotype were purified from the 3rd tumors of the same phenotype for 6th transplantsations. Note that the only 2nd ALDH\(^+\)CD44\(^-\)\(\alpha_2\)\(^+\)\(\beta_1\)\(^+\) tumor was very small, giving rise to only a total of \(\sim 20,000\) live cells, and therefore, only 4 injections were done for the 3rd generation.

\(^c\)For the first set of experiments, LAPC9 cells of various phenotypes were purified from the AI xenograft tumors maintained in castrated male NOD/SCID mice and injected s.c. in Matrigel at the indicated cell doses. In the second set of experiments, LDA were performed using purified TM-depleted and the other three cell populations indicated, the latter of which were also purified from the 3rd tumors for 6th transplantsations.
and ALDH⁺CD44⁺α2β1⁺ (TIF 1/421), were significantly more tumorigenic than TM⁻ cells (Table 1). However, as tumor cells of a specific phenotype were sorted from the 1⁻ tumors of the same phenotype (e.g., purifying TM⁻ cells from 1⁻ TM cell-derived tumors) for the 2⁻ transplantations in intact male mice, TM⁺ cells maintained the highest tumorigenicity compared with ALDH⁺CD44⁺α2β1⁺, ALDH⁺CD44⁺α2β1⁻, and TM⁻ cells (Table 1). Similarly, TM⁺ cells were greatly enriched in tumor-regenerating capacity compared with all other cell populations at the 3⁻ transplantations in intact male mice (Table 1). Notably, during serial passages, tumors regenerated from TM⁺ cells at each generation contained TM⁺ cells as the minority with the majority of cells being non-TM⁻ cells (Supplementary Fig. S3, left), suggesting that the TM⁺ cells possess self-renewal potential in vivo. Interestingly, protein levels of two well-characterized differentiation markers, i.e., AR and PSA, were significantly decreased in TM⁻ cell-derived AD tumors than TM⁺ cell-derived counterparts even after two consecutive passages in intact male mice (Supplementary Fig. S4), supporting the general notion that CSCs are less differentiated (4). Taken together, these observations demonstrate that TM⁺ LAPC9 cells are endowed with CSC (i.e., long-term tumor-propagating) properties in hormone-proficient conditions.

**TM⁺ cells can function as the cell-of-origin for CRPC and possess long-term CRPC-propagating activity in androgen-ablated male mice**

Given that TM⁺ cells are prominently enriched in AI tumors, we ponder a critical question: Can TM⁺ cells in AD tumors initiate tumor regeneration in androgen-ablated hosts? To answer this question, we purified TM⁺ and TM⁻ cells from maintenance of LAPC9 AD tumors and performed LDA by implanting different doses of cells into castrated immunodeficient NOD/SCID-Il2rg⁻/⁻ (NSG) mice. Remarkably, TM⁺ cells demonstrated ~43-fold higher tumor-initiating capacity in castrated hosts compared with TM⁻ cells (TIF 1/49 vs. 1/2,142; Fig. 2A). As few as 100 TM⁺ cells initiated 7/8 tumors, whereas 100 TM⁻ cells did not regenerate a single tumor in castrated hosts (Fig. 2A). Differences in both tumor incidence and weight between the two groups were statistically highly significant (Fig. 2A). This experiment provides direct evidence that the TM⁺ LAPC9 cells can function as the cell-of-origin for CRPC.

To determine whether the TM⁺ cells can in the long term propagate CRPC in androgen-deficient hosts, we purified TM⁺ and TM⁻ cell populations from LAPC9 AI tumors and carried out LDA and serial transplantation assays in fully castrated hosts, i.e., castrated male NOD/SCID mice also treated with bicalutamide (ref. 16; Fig. 2B and C; Table 1). In the 1⁻ generation, the TM⁺ cells demonstrated much higher tumorigenic potential than TM⁻ cells in castrated mice (TIF 1/2, 590 vs. 1/21,299, respectively; Fig. 2B; Table 1). In the 2⁻ generation tumor experiments, the TM⁺ cells, in a cell dose–dependent manner, regenerated robust AI tumors in castrated hosts such that as few as 100 cells generated 5/8 tumors, whereas the TM⁻ cells did not generate tumors at 100 and 1,000 cell doses (Fig. 2C; Table 1). Overall, the TM⁺ cells manifested significantly higher 2⁻ tumor-regenerating capacity than the TM⁻ LAPC9 cells (TIF 1/283 vs. 1/13,802, respectively; \( P = 2.23 \times 10^{-9} \); Table 1). These results suggest that the TM⁺ LAPC9 cells possess long-term CRPC-propagating capability. In full agreement with this conclusion, both primary and secondary LAPC9 AI tumors derived from the TM⁺ cells contained a similar small population (i.e., 10%–20%) of TM⁺ cells as the parental AI tumors (Supplementary Fig. S3, right), suggesting that the TM⁺ cells in AI tumors self-renewed in vivo.

To further determine the intrinsic castration resistance in TM⁺ prostate cancer cells, we sorted TM⁺ and non-TM⁺ (i.e., TM-depleted) cells from LAPC9 AI tumors, and carried out similar LDA in castrated mice. We observed that the TM⁺ cells displayed much higher tumor-regenerating activity than the isogenic TM-depleted cells (Fig. 2D; Table 1). Likewise, TM⁺ cells were able to self-renew for subsequent generations (Table 1). It was apparent that the TM⁺ cells outcompeted other subpopulations (e.g., ALDH⁺CD44⁺α2β1⁺, ALDH⁺CD44⁺α2β1⁻) with respect to tumor-initiating and tumor-propagating abilities in castrated mice (Table 1). In support of the above tumor experiments, the TM⁺ LAPC9 cells purified from the AI tumors demonstrated much higher sphere formation (Fig. 2E) and clonogenic (Fig. 2F) activities than the TM⁻ cells in “castrated” culture conditions.

Lastly, we applied similar strategies to study additional AI models. TM⁻ PC3 cells were significantly more clonogenic than TM⁺ PC3 cells in castrated culture conditions (Fig. 2G and H). Importantly, the TM⁺ PC3 cells manifested much higher tumorigenic potential than the TM⁻ PC3 cells generating more and larger tumors (Fig. 2I). Purified TM⁺ LAPC4 AI cells also exhibited increased clonogenicity as compared with TM⁻ LAPC4 AI cells in “castrated” culture conditions (data not shown). Combined, these results demonstrate that (i) the TM⁺ cells in LAPC9 AD tumors can function as the cell-of-origin for LAPC9 AI tumors, (ii) the TM⁺ cells in LAPC9 AI and PC3 models possess great CRPC-regenerating activity, (iii) the LAPC9 AI TM⁺ cells possess long-term CRPC-propagating activities, and (iv) the AI TM⁻ cells from multiple models exhibit intrinsic castration resistance in vivo.

**Untreated primary human prostate cancer (HPCA) samples have TM⁺ cells that manifest CSC properties in castrated culture conditions**

Next, we explored the potential clinical relevance of the above findings in xenograft studies using cells purified from 16 untreated HPCA samples, all of which have >75% tumor involvement (Supplementary Table S2; Supplementary Fig. S5A). Tumor areas were confirmed by robust expression of AR, PSA, and racemase and lack of expression of CK5 (Supplementary Fig. S5B). We first purified HPCA cells as the CD45⁻“Trop2” epithelial cells (22, 30), which were then analyzed for the abundance of TM⁺ cells (Fig. 3A and B). We found that all these untreated primary tumors contained TM⁺ cells ranging from ~0.001% to as high as ~14.5% (Fig. 3B). When the TM⁺ cells were purified from HPCA201 and cultured in castrated conditions (CDSS + enzalutamide), they exhibited much enhanced clonal, clonogenic, and self-renewal capacities compared with TM⁻ cells (Fig. 3C). Notably, the HPCA201 TM⁺ cells also demonstrated significantly higher secondary colony-forming activity than the corresponding TM⁻ cells (Fig. 3C, b). Purified HPCA222 TM⁺ cells generated much bigger and more clones and colonies than corresponding TM⁻ cells in castrated culture conditions (Fig. 3D). Importantly, the HPCA222 TM⁺ cell–derived colonies were positive for TMPRSS–ERG fusion (Supplementary Fig. S5C-D), indicating the tumor cell origin of the colonies. The HPCA 223 TM⁺ cells similarly demonstrated higher clonal and colony-forming capabilities than the corresponding TM⁻ cells.
Interestingly, some HPCA samples contained almost undetectable TM\(^+\) cells (Fig. 3B) but contained double- and/or single-positive cells (Fig. 3A; 19). In the HPCA samples analyzed, we observed that double-positive (i.e., ALDH\(_{hi}\)CD44\(^+\)) HPCA cells were much more clonal and clonogenic than double-negative (i.e., ALDH\(_{lo}\)CD44\(/\)C0) cells with significantly increased colony sizes and clone numbers (Supplementary Fig. S6B and S6C). However, HPCA210 ALDH\(_{hi}\) cells exhibited similar clonal and clonogenic abilities as ALDH\(_{lo}\) cells (data not shown). Taken together, these results indicate that untreated primary HPCA samples also contain TM\(^+\) cells that are intrinsically resistant to castration.

Phenotypic markers are functionally involved in CRPC development

Next, we attempted to determine whether the phenotypic markers used to define TM\(^+\) cells are causally involved in tumor regeneration under AI conditions by performing lentiviral-mediated knockdown of integrin\(_\alpha_2\), CD44, ALDH1A1, and ALDH7A1 followed by assessing the impact on tumor regeneration in castrated male NOD/SCID mice (Supplementary Fig. S7; see Supplementary Fig. S8A and S8B for knockdown efficiency). Knocking down integrin\(_\alpha_2\) in LAPC9 AI tumor cells decreased tumor incidence (5/9 or 55.6%) as compared with the control (6/6 or 100%; Supplementary Fig. S7A), whereas knocking down...
integrin α2 in LAPC4 AI tumor cells resulted in significantly smaller tumors (Supplementary Fig. S7B). CD44 knockout in LAPC9 AI tumor cells not only inhibited tumor initiation but also reduced tumor burden (Supplementary Fig. S7C). Finally, ALDH1A1 knockout in LAPC9 AI cells led to reduced spheres (Supplementary Fig. S8C) and a 50% inhibition in tumor weight (Supplementary Fig. S7D), whereas ALDH7A1 knockdown partially affected sphere-forming and tumor-initiating capacities in LAPC9 AI cells (Supplementary Fig. S8C; data not shown). Collectively, these results indicate that the phenotypic markers are causally important in imparting the castration-resistant CSC properties.

**TM⁺ prostate cancer cells have basal cell features and preferentially express CSC and castration resistance–associated genes such as RegIV and SOX9**

To further characterize the TM⁺ cells at the molecular level, we purified out TM⁺ and TM⁻ LAPC9 cells from AD and two different generations of AI tumors and performed qRT-PCR analysis of the genes associated with luminal/basal cell differentiation, castration resistance, and CSCs (Fig. 4A–C; Supplementary Table S3). This analysis revealed interesting changes in the gene expression patterns. In LAPC9 AD tumors, the TM⁺ cells overall expressed higher levels of basal cell genes (CK5, CK14, and CD44) whereas the TM⁻ cells expressed higher levels of luminal genes (AR, PSA, and CK18 with the exception of CK8; Fig. 4A). In LAPC9 AI tumors, the TM⁺ cells still expressed higher levels of basal markers CK5 and CK14 but the TM⁻ cells expressed high PSA, CD26 (31), and CK19 mRNA levels while expressing similar levels of other luminal markers (i.e., AR, CK8, CK18, and Nkx3.1) to TM⁺ cells (Fig. 4B and C).

The TM⁺ cells in the 6° generation LAPC9 AI tumors consistently expressed higher levels, compared with the TM⁻ cells, of RegIV, Sox9, and Ube2c, which have been implicated in castration resistance and CRPC progression (32–35), as well as several CSC-associated molecules, including TGFBR-1, Bcl-2, and Stat3.
Distinct microRNA (miRNA) expression profiles in TM^+^ LARC9 AI cells and CRPC-promoting effects of miR-499-5p

Our laboratory has been studying the mechanisms whereby microRNAs (miRNA) regulate PCSCs and prostate cancer development and metastasis (36–38). To understand how miRNAs might regulate the TM^+^ castration-resistant prostate cancer cells, we used a miRNA SmartChip that contained >1,000 human miRNAs to compare miRNA expression profiles of TM^+^ with TM-depleted LARC9 AI cells. We found that 59 miRNAs were significantly upregulated, whereas 22 miRNAs were downregulated in the TM^+^ cells (Fig. 5A; Supplementary Table S4). As an example to establish a cause-and-effect relationship between the differentially expressed miRNAs and castration resistance, we focused on miR-499-5p, which was among the most upregulated miRNAs in the TM^+^ cells (Fig. 5A). miR-499-5p is a relatively novel and understudied miRNA. A recent paper reported its potential oncogenic functions in colorectal cancer cells via targeting FOXO4 and PDCD4 (39). We found that miR-499-5p was expressed at higher levels in purified TM^+^ LARC9 AI cells (Fig. 5B) and in the LARC9 AI tumors (Fig. 5C). Overexpression of miR-

(Fig. 4C). Preferential expression of RegIV and SOX9 in TM^+^ cells was further confirmed in a 15^th^ generation LARC9 AI tumor (Fig. 4D). To determine whether RegIV and SOX9 are causally involved in conferring on TM^+^ LARC9 cells, certain CSC properties and castration resistance, we knocked down RegIV and SOX9 in LARC9 AI bulk cells (Supplementary Fig. S8D–S8E), which significantly attenuated their sphere-forming abilities (Fig. 4E). RegIV and SOX9 knockdown in TM^+^ LARC9 AI cells also reduced their clonogenicity in castrated culture conditions (Fig. 4F) and tumor growth in castrated mice (Fig. 4G). These data suggest that RegIV and SOX9 regulate the CSC properties of the TM^+^ LARC9 AI cells.

Distinct microRNA (miRNA) expression profiles in TM^+^ LARC9 AI cells and CRPC-promoting effects of miR-499-5p

Our laboratory has been studying the mechanisms whereby microRNAs (miRNA) regulate PCSCs and prostate cancer development and metastasis (36–38). To understand how miRNAs might regulate the TM^+^ castration-resistant prostate cancer cells, we used a miRNA SmartChip that contained >1,000 human miRNAs to compare miRNA expression profiles of TM^+^ with TM-depleted LARC9 AI cells. We found that 59 miRNAs were significantly upregulated, whereas 22 miRNAs were downregulated in the TM^+^ cells (Fig. 5A; Supplementary Table S4). As an example to establish a cause-and-effect relationship between the differentially expressed miRNAs and castration resistance, we focused on miR-499-5p, which was among the most upregulated miRNAs in the TM^+^ cells (Fig. 5A). miR-499-5p is a relatively novel and understudied miRNA. A recent paper reported its potential oncogenic functions in colorectal cancer cells via targeting FOXO4 and PDCD4 (39). We found that miR-499-5p was expressed at higher levels in purified TM^+^ LARC9 AI cells (Fig. 5B) and in the LARC9 AI tumors (Fig. 5C). Overexpression of miR-

(Fig. 4C). Preferential expression of RegIV and SOX9 in TM^+^ cells was further confirmed in a 15^th^ generation LARC9 AI tumor (Fig. 4D). To determine whether RegIV and SOX9 are causally involved in conferring on TM^+^ LARC9 cells, certain CSC properties and castration resistance, we knocked down RegIV and SOX9 in LARC9 AI bulk cells (Supplementary Fig. S8D–S8E), which significantly attenuated their sphere-forming abilities (Fig. 4E). RegIV and SOX9 knockdown in TM^+^ LARC9 AI cells also reduced their clonogenicity in castrated culture conditions (Fig. 4F) and tumor growth in castrated mice (Fig. 4G). These data suggest that RegIV and SOX9 regulate the CSC properties of the TM^+^ LARC9 AI cells.

(Fig. 4C). Preferential expression of RegIV and SOX9 in TM^+^ cells was further confirmed in a 15^th^ generation LARC9 AI tumor (Fig. 4D). To determine whether RegIV and SOX9 are causally involved in conferring on TM^+^ LARC9 cells, certain CSC properties and castration resistance, we knocked down RegIV and SOX9 in LARC9 AI bulk cells (Supplementary Fig. S8D–S8E), which significantly attenuated their sphere-forming abilities (Fig. 4E). RegIV and SOX9 knockdown in TM^+^ LARC9 AI cells also reduced their clonogenicity in castrated culture conditions (Fig. 4F) and tumor growth in castrated mice (Fig. 4G). These data suggest that RegIV and SOX9 regulate the CSC properties of the TM^+^ LARC9 AI cells.

(Fig. 4C). Preferential expression of RegIV and SOX9 in TM^+^ cells was further confirmed in a 15^th^ generation LARC9 AI tumor (Fig. 4D). To determine whether RegIV and SOX9 are causally involved in conferring on TM^+^ LARC9 cells, certain CSC properties and castration resistance, we knocked down RegIV and SOX9 in LARC9 AI bulk cells (Supplementary Fig. S8D–S8E), which significantly attenuated their sphere-forming abilities (Fig. 4E). RegIV and SOX9 knockdown in TM^+^ LARC9 AI cells also reduced their clonogenicity in castrated culture conditions (Fig. 4F) and tumor growth in castrated mice (Fig. 4G). These data suggest that RegIV and SOX9 regulate the CSC properties of the TM^+^ LARC9 AI cells.

(Fig. 4C). Preferential expression of RegIV and SOX9 in TM^+^ cells was further confirmed in a 15^th^ generation LARC9 AI tumor (Fig. 4D). To determine whether RegIV and SOX9 are causally involved in conferring on TM^+^ LARC9 cells, certain CSC properties and castration resistance, we knocked down RegIV and SOX9 in LARC9 AI bulk cells (Supplementary Fig. S8D–S8E), which significantly attenuated their sphere-forming abilities (Fig. 4E). RegIV and SOX9 knockdown in TM^+^ LARC9 AI cells also reduced their clonogenicity in castrated culture conditions (Fig. 4F) and tumor growth in castrated mice (Fig. 4G). These data suggest that RegIV and SOX9 regulate the CSC properties of the TM^+^ LARC9 AI cells.
Figure 5.

TM⁺ and TM⁻ LAPC9 AI cells have distinct miRNA expression profiles. A, Wafergen SmartChip human miRNA array was performed using TM⁺ and TM-depleted (i.e., dep) cells that were purified from serially passaged LAPC9 AI (i.e., 6⁻/C14⁻ and 7⁻/C14⁻) tumors. Differentially expressed miRNAs were selected based on $P < 0.05$. 59 miRNAs were significantly upregulated in TM⁺ cells, whereas 22 miRNAs were downregulated in TM⁺ cells. miR-499a-5₃ was highlighted by a red arrow. B, qRT-PCR validation of miR-499-5₃ expression in TM⁺ and TM⁻/C₀ cells sorted from LAPC9 AI 6⁻/C14⁻ and 15⁻/C14⁻ tumors. C, LAPC9 AI tumors display higher miR-499-5₃ expression than LAPC9 AD tumors. Shown is the bar graph (mean ± SD; $n = 3$). D, miR-499-5₃ overexpression in LAPC9 AD bulk cells promotes their clonogenic activities in castrated conditions. Bulk LAPC9 AD cells were transfected with miR-499-5₃ and negative control (NC) mimic (30 nmol/L, 48 hours), plated in 6-well ULA plates and cultured in CDSS-containing IMDM medium plus 10 μmol/L enzalutamide. Spheres were counted in 2 weeks. E, miR-499-5₃ knockdown in LAPC9 AI TM⁺ cells impairs their clonogenicity under castrated culture conditions. TM⁺ cells purified from LAPC9 AI tumors were transfected with nontargeting negative control miRNA inhibitor (anti-negative control) or miR-499-5₃ inhibitor (anti-miR-499-5₃) (20 nmol/L, 72 hours), plated in 6-well ULA plates and cultured for 2 weeks. CDSS-containing IMDM medium plus 10 μmol/L enzalutamide was used. The bar graph (mean ± SD; $n = 3$) and respective images are shown. F, overexpression of miR-499-5₃ in the TM-depleted LAPC9 AI cells promotes CRPC regeneration. The TM-depleted LAPC9 AI cells were purifed from xenografts, transfected with the negative control or miR-499-5₃ oligos (30 nmol/L), and implanted, at two cell doses, s.c. in castrated male NOD/SCID mice. Tumors were harvested 56 days after implantation. The difference in TIF was statistically significant. G, overexpression of miR-499-5₃ antagonism in the TM⁺ LAPC9 AI cells inhibits CRPC growth. The TM⁺ LAPC9 AI cells were purified from xenografts, transfected with the anti-negative control or anti-miR-499-5₃ oligos (30 nmol/L), and implanted s.c. in castrated male NOD/SCID mice. Tumors were harvested 53 days after implantation.
499-5P in LAPC9 AD tumor cells by transfection of miR-499-5P oligos promoted sphere formation compared with cells transfected with negative control oligos (Fig. 5D). In contrast, transfection of the anti-miR-499-5P oligos in the TM+ LAPC9 AI cells suppressed sphere formation (Fig. 5E). Importantly, overexpression of miR-499-5P in the TM-depleted LAPC9 AI cells promoted tumor regeneration in castrated mice (Fig. 5F), whereas overexpression of anti-miR-499-5P in the TM+ LAPC9 AI cells significantly inhibited tumor growth (Fig. 5G). Together, these results demonstrate that the TM+ prostate cancer cells express a unique miRNA profile that causally regulates their tumorigenic and castration-resistant properties.

Discussion

Two principal mechanisms, i.e., intrinsic and acquired, underlie the resistance of cancer cells to both general and targeted anticancer therapeutics (40). Intrinsic resistance implies that prior to treatments, therapy-insensitive cell preexist in the tumor and become selected and enriched during treatment, whereas acquired resistance is caused by treatment-induced gene mutations and other adaptive responses. Both mechanisms of therapy resistance have been reported in clinical tumors (40–42). Here, we provide a prototypical example for a population of cancer cells that can mediate intrinsic therapy resistance.

As early as 1981, Isaacs and Coffey, working on the rat Dunning R-3327-H prostate adenocarcinoma (H-tumor) model, performed a fluctuation analysis to show that the H-tumor relapse after ADT was due to continuous proliferative growth of preexisting AI prostate cancer cells after castration (43). Exactly what cell population in this model that became selected during castration was uncharacterized (43). In 1999, Craft and colleagues, by performing a similar fluctuation analysis, provided evidence in the LAPC9 model that ADT selected and clonally expanded the preexistent AI cells, resulting in outgrowth of CRPC (44). Nevertheless, which cell population in LAPC9 mediated the CRPC emergence was unclear. Here, we demonstrate that the TM+ cell population in the LAPC9 model can function as both a cell-of-origin as well as the tumor-propagating cell population for the established CRPC.

LAPC9 is a well-known prostate cancer xenograft expressing PSA and wild-type AR, which our laboratory has been using for the past 10 years to study PCSCs (6, 7, 16, 19, 36). Our earlier work has shown that CD44+ prostate cancer cells in several xenograft models, including LAPC9, are highly enriched in PCSCs (6). Follow-up work demonstrates that the CD44α2β1+ LAPC9 cells are even more tumorigenic than CD44+ cells (7). The current project emanated from our recent observations that the PSA+/lo cell population, which preexists in the AD tumors but becomes enriched in AI tumors, can regenerate larger tumors than PSA+ cells in fully castrated hosts (16). This study provided the first evidence for a population of preexisting human prostate cancer cells that preferentially generate AI tumors (16). Nonetheless, both PSA-/hi and PSA+ LAPC9 cells regenerated AI tumors with similar incidences (16), suggesting that only a subset of the PSA-/lo cells is actually mediating the AI regeneration. Indeed, microarray analysis led to the identification of the TM+ subset that was greatly enriched in AI tumor-regenerating activities (16).

By establishing serially passaged AI tumors and by focusing on the LAPC9 system, we have made many important and novel findings. First, the TM+ cells in AD tumors possess long-term tumor-propagating activities in androgen-proficient hosts. Second, the TM+ cells purified from the LAPC9 AD tumors can preferentially initiate xenograft tumors in fully castrated hosts, suggesting that this population can preferentially function as the cell-of-origin for AI prostate cancer. Third, the TM+ cells gradually become enriched in serially transplanted AI tumors in vivo. Fourth, the TM+ LAPC9 cells purified from the AI tumors can serially propagate the xenograft tumors in androgen-ablated hosts compared with either TM- or TM-depleted cells, suggesting that the TM+ LAPC9 cells can also function as robust CRPC-propagating cells. Fifth, the phenotypic markers used to define TM+ cells are functionally important for the TM+ cell activities. Finally, the TM+ LAPC9 cells seem to possess a unique miRNA expression profile. To our knowledge, the (serial) tumor transplantation studies presented here (Fig. 2; Table 1) represent the most comprehensive effort to define a population of human prostate cancer cells that function as the cell-of-origin as well as the tumor-propagating cells for CRPC.

Is the TM+ cell population unique to the LAPC9 model? At least in one other model, i.e., PC3, which is all PSA-, the TM+ cells exhibit significantly higher tumor-regenerating activity than the corresponding TM- cells in castrated animals. It is interesting that several other AI cell lines examined, including PPC-1, Du145, and 22Rv1, all contain significant percentages of TM+ cells, which, we believe, may also possess high AI tumor-regenerating capacities compared with the corresponding TM- cells. In several other xenograft models, including LNCaP and PC3, the fraction of TM+ cells in the AD tumors is small, which, nevertheless, also increases in the AI tumors, although the significance of the TM+ cell fraction in these models remains to be determined. Of significance, however, we have detected the TM+ cells in more than a dozen of untreated HPCA samples. The relative abundance of TM+ cells in primary HPCA samples vary widely, but freshly purified TM+ HPCA cells manifested significantly higher colony-forming abilities than the corresponding TM- cells in castrated culture conditions. Intriguingly, the ALDH1+CD44+ double-positive cells isolated from two HPCA samples also display significant castration-resistant CSC properties. Taken together, our studies in multiple culture/xenograft models and primary HPCA samples suggest that (i) the TM+ prostate cancer cell population seems to be widely present, (ii) the TM+ cells in some tumor systems, exemplified by LAPC9 and PC3, possess great tumor-initiating and tumor-propagating activities in androgen-deficient hosts, (iii) the TM+ HPCA cells in primary tumors also possess great survival advantages and colony-forming capabilities under androgen-deficient culture conditions, and (iv) in some HPCA samples, the ALDH1+CD44+ double-positive cells may behave similarly to the TM+ cells.

What are the TM+ cells? qRTPCR-based phenotyping suggests that the TM+ LAPC9 cells in both AD and AI tumors are basal-like, preferentially expressing CK5, CK14, and CD44 mRNAs and less differentiated expressing lower levels of PSA and CD26 mRNAs. This would be consistent with the fact that this subpopulation of the cells was initially uncovered from the PSA-/lo prostate cancer cell population (16). This is also consistent with our current observations that the AI cell lines, such as PPC-1, PC3, and Du145, all of which are PSA-/lo, have significantly higher percentages of the TM+ cells than the AD LAPC9, LNCaP, and LNCaP tumors, in which the majority of cells are PSA+. It should be noted, though, that cell populations that can mediate and propagate CRPC may not necessarily be all basal-like. For instance, in the BM18

Published OnlineFirst April 8, 2016; DOI: 10.1158/1078-0432.CCR-15-2956

Downloaded from clincancerres.aacrjournals.org on October 24, 2017. © 2016 American Association for Cancer Research.
xenograph model, cells that mediate CRPC prominently express luminal markers CK18 and NKX3.1 (14). What confers the TM⁺ prostate cancer cells the 'hardy' properties to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors? Part of the answer seems to reside with the three deidentities to readily regenerate and propagate the AI tumors?

Kers, i.e., CD44, integrin α2, and ALDH1A1, whose knockdown

diminished the tumor-regenerating activities of the TM⁺ cells in castrated conditions. This is not surprising considering that the CD44 and integrin α2 function as important adhesion and signaling molecules and that ALDH1A1, and perhaps ALDH7A1, may further extend cell survival by detoxification (e.g., eliminating ROS). Another reason is that the TM⁺ prostate cancer cells preferentially express genes associated with (cancer) stem cells and castration, illustrated by molecules such as Reg IV, SOX9, UBE2C, and TGFBR-1. Knocking down RegIV and SOX9 in the TM⁺ LAPC9 cells partially inhibits tumor regeneration in androgen-deficient hosts, implicating their causal functions in TM⁺ cells. Finally, the TM⁺ cells express a unique profile of miRNAs. Using miR-495-5p as an example, which is overexpressed in the TM⁺ cells compared with the TM⁻ depleted cells, we show that this miRNA is also conferring on the TM⁺ LAPC9 cells castration-resistant properties. Other microRNAs uncovered in this screening may also likely be involved in modulating the biologic properties of the TM⁺ prostate cancer cells. Collectively, the results make it clear that multiple molecules and mechanisms likely come together to endow the TM⁺ prostate cancer cells unique capabilities to function as both the cell of origin and tumor-propagating cells for CRPC.

Our recent work has illustrated that the PSA⁻__/lo prostate cancer cell population represents a therapeutic target in treating CRPC (16, 19). The current work further pinpoints the TM⁺ subset within the PSA⁻__/lo population as the driving force of CRPC emergence and propagation. Ongoing work from our laboratory is focusing on devising strategies to target the highly tumorigenic TM⁺ cell population and on elucidating the potential relationship between TM⁺ cells and several other populations of prostate cancer cells, e.g., CD166⁺ (23) and N-Cadherin⁺ (24), that may also be able to propagate castration-resistant tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: X. Chen, X. Liu, D.G. Tang

Development of methodology: X. Chen, Q. Li, X. Liu, B. Liu, D.G. Tang

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Chen, Q. Li, J. Shen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Chen, Q. Li, C. Liu, B. Liu, K. Lin, Y. Lu, H.-P. Chao, D.G. Tang

Writing, review, and/or revision of the manuscript: X. Chen, Q. Li, R. Liu, K. Rycalj, C. Jeter, K. Lin, Y. Lu, D.G. Tang

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Chen, C. Liu, D. Zhang, T. Calhoun-Davis, D.G. Tang

Study supervision: B. Liu, D.G. Tang

Other (coordinating several collaborative groups): D.G. Tang

Acknowledgments

The authors thank Animal Core for animal care and maintenance, Molecular Biology Core for help in Wafergen SmartChip human miRNA array, Histology Core for IHC studies, Ms. P. Whitney for assistance in FACS sorting and analysis, and other lab members for helpful discussions and suggestions.

Grant Support

This project was supported, in part, by grants from NIH (RO1-CA155693), Department of Defense (W81XWH-13-1-0352 and W81XWH-14-1-0575), CPRIT (RP120380), and the MDACC Center for Cancer Epigenetics (D.G.T.). X. Chen and C. Liu were supported, in part, by BOD post-doctoral fellowship PC141581 and PC121553, respectively. Both J. Shen and Y. Lu were supported by CPRIT Core Facility Support Award RP120348.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 10, 2015; revised March 15, 2016; accepted March 27, 2016; published OnlineFirst April 8, 2016.

References


Defining a Population of Stem-like Human Prostate Cancer Cells That Can Generate and Propagate Castration-Resistant Prostate Cancer

Xin Chen, Qiuhui Li, Xin Liu, et al.

Clin Cancer Res  Published OnlineFirst April 8, 2016.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-15-2956

Supplementary Material  Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2016/09/08/1078-0432.CCR-15-2956.DC1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.