Targeting Cancer Stem Cells in Castration-Resistant Prostate Cancer

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Abstract

Purpose: Clinical evidence suggests increased cancer stem cells (CSCs) in a tumor mass may contribute to the failure of conventional therapies because CSCs seem to be more resistant than differentiated tumor cells. Thus, unveiling the mechanism regulating CSCs and candidate target molecules will provide new strategy to cure the patients.

Experimental design: The stem-like cell properties were determined by a prostasphere assay and dye exclusion assay. To find critical stem cell marker and reveal regulation mechanism, basic biochemical and molecular biologic methods, such as quantitative real-time PCR, Western blot, reporter gene assay, and chromatin immunoprecipitation assay, were used. In addition, to determine the effect of combination therapy targeting both CSCs and its progeny, in vitro MIT assay and in vivo xenograft model was used.

Results: We demonstrate immortalized normal human prostate epithelial cells, appeared nontumorigenic in vivo, become tumorigenic, and acquire stem cell phenotype after knocking down a tumor suppressor gene. Also, those stem-like cells increase chemoresistance to conventional anticancer reagent. Mechanistically, we unveil that Wnt signaling is a key pathway regulating well-known stem cell marker CD44 by directly interacting to the promoter. Thus, by targeting CSCs using Wnt inhibitors synergistically enhances the efficacy of conventional drugs. Furthermore, the in vivo mouse model bearing xenografts showed a robust inhibition of tumor growth after combination therapy.

Conclusions: Overall, this study provides strong evidence of CSC in castration-resistant prostate cancer. This new combination therapy strategy targeting CSC could significantly enhance therapeutic efficacy of current chemotherapy regimen only targeting non-CSC cells. Clin Cancer Res; 22(3): 670–9. ©2015 AACR.

Introduction

Cancer stem cells (CSCs) share many characteristics with somatic stem cells, such as immortality and self-renewal. In addition to normal stem cell properties, CSCs appear to be tumor initiators and show resistance to therapies because of their quiescence. Increasing evidence indicates that CSCs are present in the end stage of disease (1). Although the cell origin of castration resistant prostate cancer (CRPC) remains controversial, several studies clearly indicate the presence of CSC in CRPC (2, 3). Despite of many potential stem cell markers identified in prostate, in human prostate cancer, the CD44+ /CD24− cells have been associated with the prostate cancer stem cell (PCSC; ref. 4). CD44 has been implicated in numerous biologic processes including cell adhesion, migration, drug resistance, and apoptosis (5–7).

Furthermore, many studies implicate CD44 in prostate cancer development and invasion in vitro and in metastatic dissemination in vivo (8, 9). However, the mechanism(s) associated with elevated CD44 in prostate cancer is largely unknown.

DAB2IP is characterized as a novel tumor suppressor in prostate cancer metastases by inhibiting epithelial-to-mesenchymal transition (EMT; refs. 10, 11). Besides, our recent study showed that DAB2IP had a critical role in suppressing stemness through modulating CD117 transcription (12). In this study, we demonstrate that loss of DAB2IP (10, 13) expression in nontumorigenic normal prostate epithelia derived from androgen receptor-negative basal cell population also increases their tumorigenicity, stemness and chemoresistance. Unlike prostate cancer cell lines which were used in previous study (12), these normal prostate epithelial cell populations exhibit CD44+/CD24− instead of CD117+ suggesting existence of another regulation mechanism. Apparently, CD44 is not only a stem cell marker correlated with prostate cancer progression but also a driver for PCSC formation in which Wnt pathway is further identified as a key underlying mechanism in modulating CD44 expression. Based on these findings, we developed a combination strategy using Wnt inhibitor and docetaxel to target both CSC and its progeny non-CSCs respectively, to significantly enhance therapeutic efficacy of CRPC.

Materials and Methods

Cell culture and reagents

PZ-HPV7 and RWPE-1 are immortalized human prostate epithelial cell line by human papillomavirus 18; PZ-HPV7 was...
Translational Relevance

Castration-resistant prostate cancer (CRPC) recognized as a lethal disease has been implied to derive from stem cell population associated with its resistance to anti-androgen therapy and chemotherapy. In this study, we demonstrate that immortalized normal human prostate epithelial cells, appeared nontumorigenic in vivo, become tumorigenic, androgen-independent, and acquire stem cell phenotypes with chemo-resistance after knocking down a novel tumor suppressor gene (i.e., DAB2IP). The clinical data also indicate an inverse correlation between the expression of DAB2IP and prostate cancer progression. We further unveil that the Wnt pathway is a key underlying mechanism that leads to CSCs. Thus, by targeting CSCs using Wnt small molecular inhibitors synergistically enhance the efficacy of conventional chemotherapy both in vitro and in vivo models. This study offers a new promising therapeutic strategy targeting CSC in CRPC therapy.

In vitro invasion and migration assay

In vitro invasion was determined in the Matrigel-based assay. Briefly, 6.5 μm polycarbonate filters of transwell (24-well insert; pore size = 8 μm; Corning) were coated with 25 μg Matrigel. The lower chambers of Transwell were filled with 600 μL of serum-free medium and the cells were plated in the upper chamber (5 x 10^4 cells/200 μL/chamber). After incubation for 48 hours, noninvading cells on the upper surface of the membrane were removed by a cotton swab and cells on the lower surface were stained with crystal violet and quantified by measuring OD at 560 nm with 96-well plates. The cell migration assay was performed with the same method except for Matrigel-coated membrane.

Prostatasphere assay

Prostate sphere growth was based on Lawson and colleagues (16). A total of 3 x 10^5 cells in PrEGM media were mixed 1:1 (v/v) with Matrigel (BD Bioscience Cat. No. 354234, 9–12 mg/mL) in a total volume of 300 μL. Each sample was subsequently plated into 24-well plates and allowed to solidify for 15 minutes, after which 1 mL PrEGM media was added. Cells were thereafter replenished every 3 days, by the removal of 0.5 mL of spent media and the addition of 0.5 mL of fresh media. Spheres were counted 14 days after plating.

Hoechst 33342 dye exclusion assay

The protocol was based on Kim and colleagues (17) with slight modifications. Briefly, cells (1 x 10^6 cells/mL) were seeded in 10-cm culture dishes allowed to attach and washed twice with PBS. Then, the cells were incubated with Hoechst 33342 (5 μg/mL, Life Technologies) in medium containing 5% FBS at 37°C for 10 minutes. After washing with PBS, cells were swiftly trypsinized and washed with ice-cold PBS. Cells were then filtered and resuspended in ice-cold PBS. Propidium iodide (5 μg/mL) was added 5 minutes before analysis to discard dead and apoptotic cells. Cells were analyzed by DakoCytomation MoFlo cytometer using dual-wavelength analysis (blue, 450/20 nm; red, 670 nm) after excitation with 350-nm UV light.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted with the RNeasy Mini Kit (QIAGEN) and 1 μg RNA was reverse transcribed with the Vilo cDNA Synthesis Kit (Life Technologies). Real-time PCR analysis was set up with the SYBR Green Supermix Kit (Life Technologies) and carried out in MyiQ thermal cycler (Bio-Rad). The relative level of target mRNA was determined by normalizing 18S RNA. All experiments were repeated at least twice to triplicate results.

Flow cytometry and fluorescence-activated cell sorting

For detection of cell surface expression of CD24 and CD44, cells were incubated with allopurinol (APC)-conjugated human monoclonal CD24 Ab (BD Biosciences) and phycoerythrin (PE)-conjugated human monoclonal CD44 Ab (BD Biosciences) for 30 minutes, and analyzed using flow cytometry (FACS Calibur, BD Biosciences). Cell sorting was performed with FACS Aria cell sorters (BD Biosciences).

Transfection and luciferase reporter assay

Cells were seeded in 24-well plates with 70% confluency before transfection. Cells were cotransfected with luciferase reporter plasmids (0.5 μg/well) and internal control, pRL-TK (Promega; 2 ng/well), expressing the Renilla luciferase. Transfections were performed using Xfect (Clontech) according to the manufacturer’s
instructions. Forty-eight hours after transfection, the wells were rinsed twice with phosphate-buffered saline, and cells were harvested with 200 μL of passive lysis buffer (Promega). Following a brief freeze–thaw cycle, the insoluble debris was removed by centrifugation at 4 °C for 2 minutes at 14,000 rpm. Aliquots of the supernatant (20 μL) were then immediately processed for sequential quantitation of both firefly and Renilla luciferase activity (Dual-Luciferase Assay System, Promega) using a Monolight TD 20/20 luminometer (Turner Designs). The activity of the Renilla reporter plasmid was used for normalization of transfection efficiency. All transfection experiments were performed in triplicates.

Western blot
Cells were washed twice with PBS and lysed in ice-cold lysis buffer [150 mmol/L NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mmol/L Tri (pH 8.0), protease inhibitor cocktail (Roche)], and cleared by microcentrifugation. Equal amounts of protein were subjected to electrophoresis on 7% or 10% NuPAGE gels (Life Technologies). Separated proteins were transferred onto nitrocellulose membranes, and membranes were incubated with 2% nonfat dry milk (w/v) for 1 hour and then washed in PBS containing 0.1% Tween 20. Membranes were then incubated with primary antibody (Ab), and Ab binding was detected using the appropriate secondary Ab coupled with horseradish peroxidase. Primary Abs used were as follows: mouse monoclonal anti-actin was purchased from Sigma, mouse polyclonal anti-GSK3β was from Santa Cruz Biotechnology, rabbit polyclonal anti-β-catenin, phospho-β-catenin (T41/S45), and phospho-GSK3β (S9) were from Cell Signaling.

Chromatin-immunoprecipitation assay
Chromatin-immunoprecipitation (ChIP) assay were performed by using the ChIP-IT Express Enzymatic Kit (Active motif, Cat. No. 53009) according to the manufacturer’s instructions. In briefly, cells were cross-linked with 1% formaldehyde for 10 minutes, quenched with glycine followed by nuclear lysis. After isolating nuclear fractions, chromatin was enzymatically sheared into 200 to 100 bp. The sheared DNA was immunoprecipitated with ChIP-grade Ab for 16 hours. After reversal of cross-linking, DNA fragments were purified on spin columns (Active motif, Cat. No. 58002). The β-catenin binding site in the CD44 promoter was determined based on combination index (CI; ref. 18).

In vitro cytotoxicity assay
Cells (5 × 10³) were seeded in 96-well plates. After 24 hours, media were changed with serum-free media for 4 hours and then different concentrations of drugs treated for 48 hours. In vitro cytotoxicity was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays according to the manufacturer's instructions (Roche). Drug synergistic effects were determined based on combination index (CI; ref. 18).

Mouse xenografts
All animal work was approved by the Institutional Animal Care and Use Committee. Dul 145 (1 × 10⁶ cells/site) cells were subcutaneously injected into 6-week-old male SCID mice. When tumors developed as measurable size, drug treatment started and tumor volume (cubic millimeters) was measured. Treatment schedules were docetaxel (5 mg/kg/ip.), LGK974 (2 mg/kg/oral gavage), and a combination of them twice a week for 3 weeks. Tumor volume was calculated by using the ellipsoid formula (π/6 × length × width × depth).

Clinical specimens, IHC, and scoring system
This study was done on the total of 194 prostate cancer specimens obtained from Vancouver Prostate Centre Tissue Bank. Seventy-six of those cases were subjected to neoadjuvant hormone therapy (NHT). The hematoxylin and eosin slides were reviewed, and the desired areas were marked. Three TMAs were manually constructed (Beecher Instruments) by punching duplicate cores of 1 mm for each sample. All the specimens were from radical prostatectomy. Tissue samples were arrayed according to Gleason score, primary or CRPC status, and with or without NHT, respectively. The Institutional Review Board of UT Southwestern and Vancouver General Hospital, BC, Canada, approved the tissue procurement protocol for this study, and appropriate informed consent was obtained from all patients.

Specimens were stained with Abs specific for CD44 (Company, 1:200) and DAB2IP (1:400) using Ventana autostainer model Discover XT (Ventana Medical System). The expression of DAB2IP or CD44 was scored based on percentage and intensity according to Allred’s scoring protocol (19). Values on a four-point scale were assigned to each specimen. The intensity score was assigned, which represented the average intensity of positive cells (0, none; 1, weak or questionably present stain; 2, intermediate intensity in a minority of cells; and 3, strong intensity in a majority of cells). All slides were scored independently by two investigators who were blinded to patient clinical information.

Statistical analysis
All error bars in graphical data represent mean ± SD. Student two-tailed t-test was used for the determination of statistical relevance between groups, and P < 0.01 was considered statistically significant. All statistical analyses were performed with GraphPad Prism software.

Results
Loss of DAB2IP transforms normal prostate epithelia into CSCs
Previous studies have demonstrated that loss of DAB2IP expression elicit EMT which have been associated with CSC development (11, 20). To determine whether loss of DAB2IP is capable of transforming nontumorigenic normal prostate epithelia derived from androgen receptor-negative basal cell population, two well-characterized cell lines (21) were used. The clonogenic ability was significantly increased in both RWPE-1 knockdown (KD) and PZ-HPV7 KD cells (Supplementary Fig. S1A). Data from soft agar assay showed a significant increase in anchorage independent growth of KD cells (Supplementary Fig. S1B) and PZ-HPV7 KD cells became tumorigenic in vivo (10).

Noteworthy, both KD cells increased stemness properties. For example, RWPE-1 KD or PZ-HPV7 KD cells were able to form spheroid prostaspheres (Supplementary Fig. S1C), but the majority of Con cells failed to form measurable prostaspheres in semisolid culture for 2 weeks. These KD cells exhibited significant sphere-forming ability in both numbers (Supplementary Fig. S1C, left) and size (Supplementary Fig. S1C, right). In addition, the side population (SP) associated with drug efflux capacity measured by Hoechst 33342 dye exclusion is commonly used for characterizing hematopoietic stem cells and CSCs (22). As shown in
Supplementary Fig. S1D, the percentage of SP increased significantly in both RWPE-1 KD and PZ-HPV7 KD cells. In addition, loss of DAB2IP significantly increased in vitro migration and invasion (Supplementary Fig. S2A and S2B). Together, these results suggest that KD cells have acquired stem cell phenotypes.

PCSC cells exhibit enriched CD44⁺/CD24⁻ populations

To identify specific stem cell markers associated with these KD cells, we screen several stem cell markers, and the results showed the consistent elevation of CD44 mRNA expression and reduction of CD24 mRNA expression in both RWPE-1 and PZ-HPV7 KD cell (Fig. 1A, Supplementary Fig. S3A and S3B), whereas the expression pattern of other CSCs surface markers was varied. Flow cytometric analysis (Fig. 1B) also demonstrated that the CD44⁺/CD24⁻ populations were significantly higher in both KD cells, whereas most Con cells exhibit CD44⁻/CD24⁺ expression suggesting that DAB2IP is a potent suppressor for stemness phenotype acquisition. To find the driving force of CSCs, we more focused on elevated CD44 rather than depleted CD24, and interestingly, PZ-HPV7T cells (13), a tumorigenic subline of PZ-HPV7 cells, D, representative IHC staining of DAB2IP and CD44 in clinical specimens (left). The scale bar represents 100 μm. Right: DAB2IP and CD44 staining score in primary prostate cancer were compared with the metastatic tissue.

Wnt signal pathway mediate the regulation of CD44 by DAB2IP

Mechanistically, the presence of DAB2IP was able to inhibit CD44 gene transcription (Supplementary Fig. S4A) that CD44 gene promoter activity increased in both KD cells compared with their Con cells, respectively. To reveal the possible mechanism of DAB2IP in regulating CD44 transcription, the structural–functional relationship of DAB2IP that contains several unique domains with distinct functional role (11) was carried out. As shown in Fig. 2A, the C2 domain of DAB2IP is the key domain in suppressing CD44 gene promoter activity. It is known that C2 domain in DAB2IP can recruit PP2A to activate GSK3β that is able to inhibit β-catenin/Wnt signaling (11). Indeed, in both RWPE-1 and PZ-HPV7 KD cells, activities of β-catenin and the downstream gene were activated by measuring Wnt-specific gene reporter activity (i.e., TOP; Fig. 2B, left and C). Consistently, PZ-HPV7T cells with elevated CD44 and CD44⁺ population sorted from both PZ-HPV7T and Du145 cells (Fig. 2B, right). Furthermore, analyses of several prostate cancer clinical data demonstrated a positive correlation between β-catenin and CD44 expression (Supplementary Fig. S4B, S4C, and S4D). The data support the role of β-catenin/Wnt signaling in regulating CD44 gene expression.
transcription. However, treatment of the AR inhibitor could not change the CD44 expression in both cell lines suggesting CD44 was regulated in AR-independent pathway (Supplementary Fig. S5). Besides, the effect of DAB2IP did not limited to specific CD44 isoforms but all isoforms’ expression responded in a same pattern (Supplementary Fig. S6). To further confirm the regulation mechanism through Wnt pathway, the effect of Wnt inhibitors such as LGK974 and IWP-2 on CD44 expression in KD cells was examined. As shown in Figure 2D, the relative transcriptional activities of CD44 in KD cells significantly decreased by the treatment of both inhibitors. Treatment of Wnt inhibitors also decreased both sphere formation (Supplementary Fig. S7A) and expression of CD44 in KD cells (Supplementary Fig. S7B). In contrast, the expression of constitutively active (CA) β-catenin mutant (S37A) in Con cells increased CD44 gene promoter activity (Supplementary Fig. S8A) and in vitro cell migration and invasion in presence of DAB2IP (Supplementary Fig. S8B and S8C). In KD cells, by reconstituting PP2A-WT or GSK3β (WT or CA) with DAB2IP could suppress CD44 gene promoter activity but PP2A-LP (catalytic inactive) failed to have the same effect (Supplementary Fig. S8D). Also, the treatment of PP2A inhibitor Okadaic acid (OA) increased CD44 gene promoter activity in a dose-dependent manner (Supplementary Fig. S8E). To demonstrate the direct interaction between β-catenin and CD44 gene promoter region, ChIP data (Fig. 3A) clearly showed that the direct binding of β-catenin to several CD44 gene promoter regions, based on the predicted β-catenin binding consensus sequences, in PZ-HPV7 KD cells. Also, ectopic expression of CA β-catenin in both PZ-HPV7 Con and 293 cells increased its binding to CD44 gene promoter (Fig. 3A–C) and enhanced CD44
expression in RWPE-1 and PZ-HPV7 Con cells (Fig. 3D). Taken together, these results conclude the direct effect of Wnt-elicited-β-catenin on CD44 gene expression in PCSCs.

CD44 drives PCSC associated with chemoresistance

Despite CD44 is known as a stem cell marker, it is also a receptor for hyaluronic acid and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (5). Thus, to determine whether CD44 is a key driver for PCSC associated with chemoresistance, CD44 expression was knock-downed using shRNA. Data (Fig. 4A) indicated that CD44 KD significantly decreased in vitro tumorigenicity and sphere formation in PC-3 and 22Rv1 cells (Fig. 4B and C). Also, decreased CD44 expression could sensitize these cells to docetaxel treatment (Fig. 4D). All together, these data support the potency of CD44 in facilitating the onset of PCSC and increasing its chemoresistance.

CRPC therapy targets CSC and its progeny

Docetaxel is the first line of chemotherapy for the CRPC patients who had unsuccessful androgen deprivation therapy (23). Nevertheless, CRPC develops to its resistant status very rapidly; it is believed that docetaxel can only kill proliferative progeny cells derived from CSCs, but fails to eradicate CSC (24). As shown in Fig. 5A, enriched CSC of RWPE-1 KD or PZ-HPV7 KD cells showed significant resistance compared with their Con cells treated with docetaxel. Nevertheless, RWPE-1 KD and PZ-HPV7 KD cells were slightly more sensitive to LGK974 than their Con cells (Fig. 5A, right). Similarly, CD44+ cells sorted from PZ-HPV7T showed higher resistance to docetaxel but more sensitivity to LGK974 than CD44- cells (Supplementary Fig. S9A). These results prompt us to explore a new therapeutic strategy by combining Wnt inhibitor with docetaxel to target CSC and its progeny cells. Indeed, RWPE-1 KD and PZ-HPV7 KD cells treated with combination regimen exhibited a synergistic effect (Fig. 5B). As expected, CD44 mRNA expression level was decreased in these KD cells treated with LGK974 alone or combination, but not docetaxel alone (Fig. 5C). Consistently, LGK974 alone or combination treatment significantly decreased the prostasphere formation of KD cells, but docetaxel failed to have any effect (Fig. 5D). Similar results were observed from IWP-2 alone or in combination with docetaxel treatment (Supplementary Fig. S9D and S9E) with the similar pattern of decreasing CD44+ cell population (Supplementary Fig. S9F). However,
constitutive overexpression of CD44S (25) in RWPE-1 and PZ-HPV7 KD cells could overcome the effect of Wnt inhibitors and show increased cell viability (Supplementary Fig. S10). These results support the hypothesis that to eradicate cancer completely, we must simultaneously target cancer-initiating cells and their progeny cell.

Figure 4.
CD44 is critical for PCSC development and its chemoresistance. A, characterization of shCD44 sublines of PC-3 and 22Rv1 cells generated using shRNA (Origene, TG34080) transfection by qRT-PCR and Western blot analyses. B, clonogenic assay of shCD44 or shvec cells were seeded in six-well plates at a density of 500 cells per well and cultured for 10 days then stained with crystal violet. The relative number of colony was determined by measuring OD560 nm. C, prostaspheres assay was performed for 2 weeks and the numbers of prostaspheres were compared in shvec and shCD44 cells. D, cells were seeded in a 96-well and treated with docetaxel for 48 hours. Cell viability was assessed by MTT assay.

Figure 5.
Wnt inhibitor reduces chemoresistance of KD cells to docetaxel. A, cells were treated with docetaxel or LGK974 for 48 hours and subjected to MTT assay. B, cells were treated with 1 nmol/L docetaxel, 100 nmol/L LGK974, or combination; and cell viability was determined 48 hours after treatment by MTT assay and drug synergistic effects were determined based on combination index (CI). CI < 1, synergistic; CI = 1, additive; CI > 1, antagonistic effect. NT, nontreatment; DCT, docetaxel; LGK, LGK974; D+L, docetaxel and LGK974 combination treatment. C, the expression levels of CD44 mRNA were analyzed 48 hours after treatment by qRT-PCR. D, the prostasphere formation was determined from cells after 24-hour treatment then plated into sphere culture condition for 2 weeks. Media containing each drug were changed every 3 days.
We further evaluate the in vivo effect of this combination strategy using CRPC model of Du145 cells which exhibit high percentage of CD44+ population. The effect of combination treatment on Du145 cell line was determined in vitro and result showed the consistent results with KD cells (Supplementary Fig. S11). Then, the mice bearing DU145 tumors were treated with docetaxel alone, LGK974, or combination of docetaxel with LGK974. Remarkably, mice treated with the combination treatment targeting both CD44+ population and their progeny CD44− fast growing cells showed a robust inhibition of tumor growth compared with mice treated with single agent (Fig. 6A and B). We noticed decreased CD44 and β-catenin levels in tumors harvested from LGK974 or combination treatment, whereas docetaxel treatment led to an increased CD44 expression, which further validated the outcome of targeted therapy (Fig. 6C and D). Taken together, Wnt inhibitor can suppress CSC population and synergize the effect of conventional therapeutics on eradicating proliferative CSC progeny in CRPC.

**Discussion**

Human primary prostate cancer is a typical androgen-dependent (AD) disease, and most of tumor cells express differentiated luminal cell markers such as CK8 and 18, but are absent of basal cell markers (26). Androgen deprivation therapy (ADT) is the gold standard regimen for metastatic prostate cancer patients. Despite the initial response, most patients eventually relapse and progress to CRPC, chemotherapy is the only option for these patients. However, only half of the patients respond to treatment and even those who initially respond to treatment eventually become resistant (26). It appears that CRPC exhibits many similar phenotypes of stem cell (27), suggesting that clonal expansion of PCSC population and/or de-differentiation of prostate cancer cells. It is believed that CSCs share many similar characteristics with normal stem cells; however, they acquire additional malignant properties, such as uncontrolled division of progeny cell, invasion/metastasis, and chemo-resistance, which eventually lead to the mortality of cancer patients (28). New therapeutic regimens have greatly improved the survival of prostate cancer patients; however, the relapse of chemoresistant tumor remains the major obstacle of complete cure of prostate cancer (29). In general, most therapeutic regimens only target the proliferative tumor cells, as the progeny cell from quiescent CSC population still remaining intact. Consequently, the expansion of CSC population results in drug resistance (30). Understanding the underlying mechanisms associated with CSC and chemo-resistance could lead to new strategies for targeting CSC.

Loss of DAB2IP is frequently detected in high-grade and metastatic prostate cancer tissues and correlated with biochemical recurrence-free survival of prostate cancer patients (11, 31). Recent data (11) indicate that DAB2IP is able to intervene EMT as an initial step of prostate cancer metastasis. EMT is associated with embryo implantation, embryogenesis, and organ development; however, it symbolizes de-differentiation of differentiated neoplastic cells. Apparently, loss of DAB2IP in prostate epithelia also enhances PCSC phenotypes associated with enriched CD44+ cell population. CD44 is a receptor for hyaluronic acid and commonly expressed in embryonic, hematopoietic, mesenchymal stem cells (32, 33). In prostate cancer cells, CD44 has been implicated as a CSC marker and enriched CD44+ cells were more tumorigenic and metastatic than CD44− cells (33). Elevated CD44 in prostate cancer has been also implicated in cancer cell proliferation, tumorigenicity, migration, invasion, and metastasis (34). We provide further evidence for the functional role of CD44 in maintaining CSC phenotype and the underlying mechanism of DAB2IP as a potent modulator for CD44 gene expression that is also supported by clinical data. CD44 comprises two kinds of exons, constant and variable ones. Former encode the
extracellular globular part (exons 1–5), a short stem as connection to the cell membrane (exons 16 and 17) and the transmembrane domain (exon 18). Exons 19 and 20 are subject to alternative splicing creating either a short or more often a long cytoplasmic tail (35). The exons 6 to 15 are variable (v1-10), enlarging the stem on its distal site and forming several distinct CD44 isoforms, referred to as CD44 variants (CD44v1-10; ref. 35). However, whether CD44s or CD44 variants is a critical CSCs marker is still under debate. Lacking all variable exons, CD44 standard (CD44s) is the most ubiquitous isoforms, and the expression pattern of the different variants of CD44 varies during lineage commitment. For example, epithelial cells express CD44v8 and CD44v6 is upregulated in monopoiesis and downregulated in granuloipoiesis (36). Besides, CD44v6 is found to confer metastatic behavior to non-metastatic tumor cells, so associated with prostate cancer metastasis and chemo-/radioresistance (37, 38). In this study, we also made an effort to find the key driver which was involved in CSCs regulation, but DAB2IP equally regulated CD44 variants with loss of DAB2IP, altered all types of CD44 variants including CD44v3, CD44v6, and CD44v8.

The mechanism(s) regulating CD44 gene expression in prostate cancer cells is largely unde (6, 39–41). In other cancer types, transcriptional factors such as Egfr-1 and AP-1 are known to regulate CD44 expression (42, 43). However, we could not validate in prostate cancer cells (data not shown). Here, we demonstrate a new mechanism of CD44 gene regulation by Wnt in which a direct binding of β-catenin-TCF/LEF complex to its gene promoter in prostate cancer. Wnt signaling has been implicated in regulating stem cells from a variety of tissues (44, 45) and its dysregulation is associated with various cancers (46). Several studies indicated that an increased nuclear β-catenin is often detected in advanced stage of prostate cancer (47) and CD44+ cells are found to predominate in visceral metastases (9). Also, a recent study indicated that the interaction between prostate cancer and bone which resulted in the resistance to androgen deprivation is mediated by bone stroma-derived Wnt5a (48). Collectively, CD44+ in prostate cancer cells may be responsible for chemoresistant CRPC, thus targeting Wnt appears be a viable therapeutic option in CRPC patients.

It is known that CSCs are more resistant to therapies because of the inherited survival advantage of CSCs from increased antiapoptotic or and drug efflux machinery (49). Emerging studies indicate the onset of CSC associates with lethal phenotypes of many types of cancer. Thus, identifying therapeutic target to eradicate CSC becomes an urgent task to improve therapeutic outcome. Although Wnt inhibitors are able to decrease CD44+ population, they fail to achieve significant therapeutic efficacy because the tumor mass population majority are proliferative CSC progeny cells. Conventional drugs such as docetaxel could target this population, thus combining both agents is likely to generate a more potent effect; indeed, our data offers a new therapeutic strategy that combination of CSC and non-CSC targeting agents produce a synergistic anticancer efficacy.

Together with our recent report (12), DAB2IP is a critical modulator in the differentiation of prostate cancer cells; loss of DAB2IP in prostate cancer could elicit de-differentiation of prostate cancer cells toward stemness phenotypes. Most encouraging, the outcome of this experimental therapeutic model provides the immediate clinical translation of a potential targeted therapy for CRPC patients because this oral-bioavailable LGK974 with a wide-spectrum inhibition of Wnt pathway is under clinical trials for cancer (50).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: E.-J. Yun
Development of methodology: E.-J. Yun
Acquisition of data (proposed animals, acquired and managed patients, provided facilities, etc.): E.-J. Yun, E. Hernandez, M. Gleave
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.-J. Yun, J. Zhou, C.-J. Lin, M. Gleave
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Prostate Cancer Stem Cell Therapy


