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SHORT COMMUNICATION

Androgen receptor expression in prostate cancer stem cells: is there a conundrum?

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Abstract Androgen deprivation therapy (ADT) is standard frontline therapy for metastatic prostate cancer. However, prostate cancer progresses to a castrate-resistant state. The response of prostate cancer to androgen deprivation is mediated by the androgen receptor (AR). Castrate-resistant disease is marked by a gain-of-function in AR and AR reactivation. The stem cell hypothesis of cancer would therefore predict that AR should be expressed in the prostate cancer stem cell, since genetic selection for gain-of-function changes in AR, such as AR gene amplification, should occur at the level of the stem cell population. Initial reports characterizing prostate cancer stem cells suggest that AR is not expressed in this population, which is an apparent conundrum. Here, we examined the CD44+/24− LNCaP putative stem cell population by in-cell Western and show that AR is expressed at the protein level. Our findings suggest that at least a subset of prostate cancers express AR in the putative stem cell population.

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Introduction

Metastatic prostate cancer is first treated with ADT by either surgical castration or medical castration with gonadotropin-releasing hormone agonists [1]. The AR is expressed in almost all prostate cancers and dictates the response of prostate cancer to ADT [2]. However, the response to ADT in the metastatic setting is transient and tumors progress to castrate-resistant prostate cancer (CRPC). A key feature of CRPC is reactivation of AR by a variety of mechanisms, such as AR gene amplification [3]. The end result is reactivation of androgen-responsive genes involved in growth and survival [4–6]. Therefore, gain-of-function changes in AR, such as gene amplification, are critical for progression to CRPC. Genetic mechanisms of resistance to hormonal therapy in prostate cancer are therefore not unlike mechanisms of resistance to chemotherapeutic agents that are inhibitors of metabolism (e.g., methotrexate), which also involve gain-of-function changes (e.g., dihydrofolate reductase gene amplification) [7].

Cancer stem cells are emerging as being important in tumor maintenance, proliferation and recurrence and are coming to the forefront as targets of therapy. Cancer stem cells left behind after systemic therapy may lead to recurrent disease. Since AR is the relevant target with ADT, determining expression of AR in prostate cancer stem cells is of paramount importance. Androgen receptor expression in stem cells would imply that these cells are directly responsive to ADT. Given that there is selection for gain-of-function changes in AR in prostate cancer, AR expression in the stem cell population, which gives rise to the diverse tumor population, would be expected. However, initial studies suggest that AR is not expressed in prostate cancer stem cells [8, 9]. It is difficult to reconcile the facts that gain-of-function changes in AR are found in CRPC and

that selection for these gain-of-function changes must occur in stem cells, which do not express AR and are not directly responsive to ADT. This is the castrate-resistant conundrum [10].

To further study this issue, we examined LNCaP cells, the most widely studied prostate cancer cell line in the laboratory. We examined AR expression at the protein level in LNCaP cells that are CD44+/CD24−, which is a putative stem cell phenotype and is enriched for tumorigenic cells [11].

Materials and methods

Flow cytometric analysis and separation

LNCaP cells were grown in RPMI media with 10% FBS, PC3 cells were grown in F-12 K with 10% FBS and 293 cells were grown in DMEM with 10% FBS. LNCaP cells were detached with trypsin, washed once in FACs buffer (PBS containing 1–2% BSA and 5 mM EDTA), then stained with anti-CD24-FITC (Invitrogen, Carlsbad, CA) and anti-CD44-PE (Invitrogen,Carlsbad, CA) using 10 µl of antibody/10^6 cells and incubated at 4°C for 15 min. Following incubation, cells were washed once with FACs buffer. For flow cytometric sorting cells were re-suspended in FACs buffer at 20×10^6 cells/ml and separated on either an Aria cell sorter (BD Biosciences, San Jose, CA) or a MoFlo High Performance cell sorter (Dako Cytomation, Carpinteria, CA). LNCaP cells were sorted into a CD44+/24− (putative stem cell) population and a population depleted of the CD44+/24− population.

In-cell Western analysis

LNCaP CD44+/24−, LNCaP depleted, PC3 and 293 cells were plated in triplicate on poly-L-lysine coated 384 well plates in DMEM:F12 plus 10 ng/ml bFGF, 20 ng/ml EGF, 5 µg/ml insulin and 0.4% BSA at 2,500 cells per well and incubated overnight. The following day, cells were fixed with 4% formaldehyde for 4 min, washed in a solution of 0.5% fish gelatin and 0.1% triton X-100 and incubated in this same solution to block for 90 min. Cells were then incubated with rabbit anti-AR (Santa Cruz, Santa Cruz, CA) and mouse anti-actin (Abcam, Cambridge, MA) in a solution of 1% fish gelatin for 2 h. After washing with PBS+ 0.1% Tween 20, cells were incubated with IRDye 800CW labeled donkey anti-mouse antibody and IRDye 680CW labeled donkey anti-rabbit antibody (LI-COR Biotechnology, Lincoln, Nebraska) for 2 h. After washing with PBS+ 0.1% Tween 20, plates were allowed to air dry and scanned with the LI-COR Odyssey Infrared Imaging System. Controls for all cells were stained with secondary antibody only and this background was subtracted from the signal. Androgen receptor signal was normalized to actin as a control for each cell type.

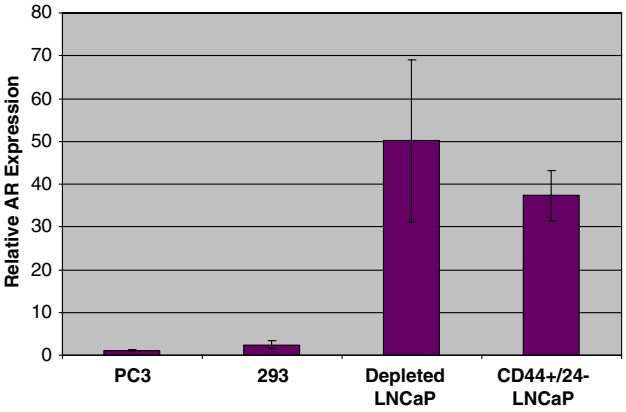

Fig. 1 CD44+/24− LNCaP cells express AR protein. AR protein expression relative to actin expression was determined in triplicate. The mean value is shown, with error *bars* representing one standard deviation from the mean. AR expression levels in CD44+/24− LNCaP cells is the same as in the depleted LNCaP population. PC3 and 293 cells were used as negative controls

Results and discussion

Flow cytometry of LNCaP cells showed that 0.05% of the entire population was CD44+/24−. The low proportion and yield of CD44+/24− LNCaP cells precluded traditional protein lysate analysis by Western blot and we overcame this by doing in-cell Westerns. The mean AR protein expression level in CD44+/24− LNCaP and LNCaP depleted populations did not differ significantly. Relative to both populations of LNCaP cells, PC3 and 293 negative controls cells did not express AR protein (Fig. 1).

Stem cells are thought to give rise to the entire spectrum of prostate epithelial tumor cells. Cancer therapies, whether they may be chemotherapeutic, signal transduction inhibitors or hormonal therapies, should be employed with an understanding of an expression of their respective targets in the stem cell population. ADT causes tumor regression in the vast majority of prostate cancers. In CRPC, gain-of-function changes occur in AR. Direct selection for cells with AR reactivation should therefore only occur in cells that express AR. The lack of AR expression in prostate cancer stem cells reported with other studies would suggest that the selection for gain-of-function changes in AR occur indirectly [10]. Here, we show that at least some prostate cancer stem cells express AR, suggesting that direct selection for AR gain-of-function may occur at the level of the stem cell. Further study of the interface of AR biology, castrate-resistant disease and prostate cancer stem cells will be required to better understand how to overcome castrate-resistant disease.

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