Asian Journal of Andrology (2013) 15, 83–84

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RESEARCH HIGHLIGHT

Identification, characterization and targeting of Docetaxel-resistant prostate cancer cells

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Asian Journal of Andrology (2013) **15,** 83–84; doi:10.1038/aja.2012.133; Published online: 3 December 2012

Men with castration-resistant prostate cancer exhibit resistance to chemotherapeutic agents such as Docetaxel. Defining the mechanisms of resistance to Docetaxel is critical for treating advanced disease. In a new study, Carlos Cordon-Cardo and colleagues determine that Docetaxel-resistant prostate cancer cells rely on the Notch and Hedgehog signaling pathways for their survival. These findings provide a rationale for the inhibition of Notch and Hedgehog pathways in Docetaxel-resistant prostate cancer. The authors demonstrate that Docetaxel-resistant cells can propagate tumors and may exist prior to treatment, suggesting that Notch/Hedgehog pathway inhibition in combination with Docetaxel may prevent treatment-resistance.

Androgen deprivation therapy (ADT) is the first line of treatment for patients with advanced prostate cancer. ADT includes orchiectomy, luteinizing hormone-releasing hormone antagonists and anti-androgens.[1] While ADT is initially beneficial, the disease commonly recurs in its lethal form originally referred to as hormone-refractory prostate cancer and now called castration-resistant prostate cancer (CRPC).[1]

Current therapeutic strategies to target CRPC include taxanes (Docetaxel, Cabazitaxel), bisphosphonates or the newer drug Denosumab.[2] Docetaxel blocks mitotic spindle assembly thus inhibiting mitosis and cellular division. This therapy targets rapidly dividing cells which is relatively specific for proliferating cancer cells while sparing quiescent normal tissues. Chemotherapy with Docetaxel in combination with other agents exhibits limited efficacy in the treatment of CRPC with an average increase in median survival of 1–2 months.[3–5] The mechanisms through which prostate cancer cells acquire chemotherapy resistance are still unclear. Identifying these mechanisms could lead to new promising strategies to overcome treatment-resistant advanced prostate cancer.

In a recent issue of *Cancer Cell*, Domingo-Domenech *et al.*[6] identified and characterized a subpopulation of Docetaxel-resistant CRPC cells. The initial studies were performed in two cell lines, DU145 and 22Rv1. DU145 is a hormone insensitive cell line, originally derived from a prostate cancer brain metastasis that has undergone numerous passages in cell culture *in vitro*. 22Rv1 is a human prostate cancer cell line that has been maintained as a xenograft tumor, serially passaged in mice. These prostate cancer cell lines show the capacity to grow in castrated mice, modeling CRPC. Both cell lines were subjected to Docetaxel treatment followed by gene expression profiling. The authors found that Docetaxel-resistant CRPC cells express very low levels of luminal and basal differentiation markers, such as cytokeratins (CK), pan-HLAI-antigens and the classical marker of prostate cancer, prostate-specific antigen (PSA) (**Figure 1a**).

After Docetaxel treatment, prostate cancer cell lines also exhibited upregulation of Notch and Hedgehog, two pathways implicated in normal prostate development (**Figure 1a**). An analogous population of CK-negative, HLAI-negative, Notch and Hedgehog positive CRPC cells could be identified in human prostate cancer patient tissues. Patients with metastatic prostate cancer had a higher percentage of CK-negative CRPC cells, in comparison to patients with primary prostate cancer. The highest percentage of CK-negative CRPC cells was observed in patients previously treated with Docetaxel.[6] The number of Docetaxel-resistant CK-negative CRPC cells positively correlates with tumor aggressiveness and negatively correlates with disease relapse, further demonstrating the clinical relevance of such cells. Domingo-Domenech *et al.*[6] went further to demonstrate that the Docetaxel-resistant CK-negative phenotype is pre-existing prior to treatment.

Many studies over the last decade have suggested the existence of a small population of cells within solid tumors that have high proliferative capacity and self-renewal ability. In prostate cancer, these cells are predicted to be castration-resistant and demonstrate the ability to generate tumors upon transplantation into mice. Using long termed cultured cell lines, previous studies have identified populations of cells with low or no PSA ($PSA^{-/lo}$)[7] expression of CD44,[8] or other markers[9] enriched for the capacity to propagate tumors. However, enriched cells directly isolated from primary clinical prostate cancer specimens capable of propagating tumors into mice have yet to be reported.

Domingo-Domenech *et al.*[6] exploited the HLAI-negative cell surface phenotype to enrich CK-negative Docetaxel resistant cells. The HLAI-negative subset from DU145 and 22Rv1 cell lines was 2000-fold more tumorigenic when compared to $HLAI^{+}$ cells[6] upon transplantation into mice (**Figure 1b**).

In this study, the authors demonstrated for the first time the existence of a defined subpopulation of cells from primary human prostate cancer specimens with tumor initiating capacity. In four out of 30 clinical specimens tested, the HLAI-negative cells could propagate tumors into mice. The low efficiency of

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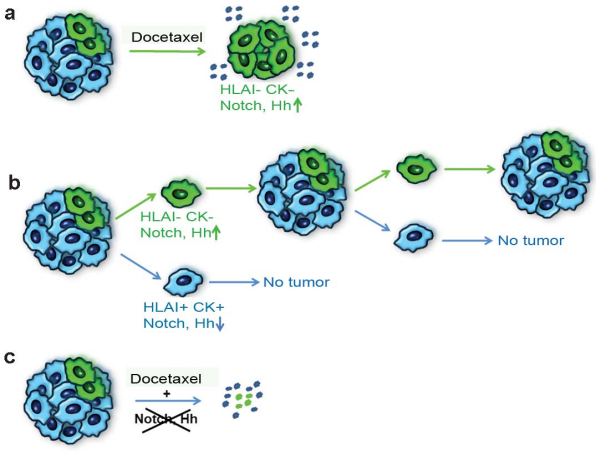

Figure 1 Isolation and characterization of Docetaxel-resistant prostate cancer cells. (**a**) Cells preferentially surviving Docetaxel treatment express low levels of pan-HLAI-antigens and CK and high levels of active Notch and Hedgehog signaling. (**b**) Prostate cancer cells with a Docetaxel-resistant phenotype can serve as tumor initiating cells. (**c**) Docetaxel-resistant prostate cancer cells are dependent on Notch and Hedgehog signaling. CK, cytokeratins.

tumor propagation may reflect the indolent nature of primary prostate cancer and suggests the need to improve the xenotransplantation assay (**Figure 1b**).

Functional studies revealed that the phenotypic Docetaxel-resistant CK-negative HLAI-negative population does not only show activated Notch and Hedgehog signaling, but it is also dependent on these two pathways. Previous studies have pointed to the therapeutic potential of targeting the Notch pathway in Docetaxel-resistant prostate cancer cells.[10,11] Domingo-Domenech *et al.*[6] take these studies further by demonstrating that downregulation of both Notch and Hedgehog pathways through combined siRNA treatment or chemical inhibitors depletes Docetaxel-resistant prostate cancer cells (**Figure 1c**). The therapeutic efficacy of combining Docetaxel with Notch and Hedgehog inhibitors was further demonstrated *in vivo*. Docetaxel showed great suppression of tumor growth only when combined with Notch and Hedgehog inhibitors in comparison to each inhibitor alone (**Figure 1c**).

These findings could potentially lead to development of new combined therapies for treatment of chemotherapy-resistant advanced prostate cancer. As an alternative strategy, the triple combination of Notch and Hedgehog signaling inhibition with Docetaxel could be more efficacious to simultaneously target both Docetaxel-sensitive and pre-existing Docetaxel-resistant cells in a chemotherapy-naive patient population. Such a strategy may be efficacious in a wide range of tissues, as combined inhibition of Notch and Hedgehog signaling has been demonstrated to sensitize chemotherapy resistant malignant glioma cells to temozolamide.[12]

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