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RESEARCH HIGHLIGHT

PSA-negative/low prostate cancer cells: the true villains of CRPC?

David J Mulholland

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Prostate cancer remains a leading cause of death in men in the United States.[1,2] Treatment for advanced disease almost always includes androgen deprivation therapy (ADT)[3] largely because the androgen receptor (AR) is expressed at high levels at many stages of disease progression. The clinical response to ADT typically includes a reduction in overall tumor burden and reduced serum levels of the AR target, prostate-specific antigen (PSA).[4]

Despite the effectiveness of recently developed inhibitors of AR signaling, many patients still progress to the lethal phenotype of castrate-resistant prostate cancer (CRPC). This sequence of events implies that minor populations of prostate cancer cells have qualities that allow them to survive front line therapy with the potential to propagate recurrent primary or metastatic disease.

Despite the considerable attention that the AR signaling pathway continues to receive as a therapeutic option for advanced disease, several lines of evidence suggest that the true villains of CRPC may be those cells with low AR signaling. First, both AR and PSA expression patterns are heterogeneous and in some instances undetectable by immunohistochemistry both in primary and metastatic disease.[5–8] Second, while the anti-androgen drug, MDV3100, was shown to reduce serum PSA levels in most patients during a in a Phase 1–2 study (62% in chemotherapy naive patients and 51% for chemotherapy-treated patients),[9] some patients either failed to respond or did not show appreciable reductions in PSA. Third, prostate cancer patients with tumors containing >50% PSA-positive cells have longer survival and more advanced tumors contain fewer PSA-positive cells.[8,10] These observations underscore the notion that prostate cancers are not only histologically heterogeneous but are likely highly heterogeneous with respect to dependency upon AR signaling and its gene targets.

In a recent publication in *Cell Stem Cell*, Qin *et al.*[11] address this possibility using an elegant method to isolate both PSA-high and PSA-neg/low expressing cell populations from the LNCaP and LAPC9 human prostate cancer cell lines. The authors carry out thorough characterizations of both cell populations with respect to gene expression patterns, stem cell qualities, tumorgenicity and therapeutic resistance, while drawing some striking parallels to primary tumor pathology.

First, the authors document the prevalence of undifferentiated PSA-neg/low tumor cells most notably in high (9–10) Gleason grade tumors with a significant portion of CRPC tumors completely lacking PSA-expressing cells. While compelling evidence suggests that AR amplification may facilitate cell survival in environments of reduced androgens,[3] the present study supports the possibility that reduced AR signaling and PSA expression is a survival response to escape ADT.

ADT poses a significant cellular stress to both normal and transformed prostate cells. Indeed, in comparing PSA-neg/low cells with PSA-high cells, the authors observed significant upregulation of antistress genes in categories of detoxification, hypoxia-responsive, p53 signaling and DNA-damage sensing. These data suggest that PSA-neg/low cells could also have greater resistance to other therapy-induced stresses beyond ADT including radiation, chemotherapy and cytotoxic exposure. However, further validation will be necessary to ascertain whether such poorly differentiated regions of primary tumors retain such qualities. Certainly, isolation of such cell populations using flow cytometry combined with functional *in vitro* tests will help elucidate this.

Cancer-initiating cells, including those with the capacity to initiate CRPC, are thought to have qualities of stemness. While not always agreed upon, the cancer stem cell hypothesis indicates that subsequent to therapy, a minor population of transformed stem cells has the ability to remain quiescent during remission which may mobilize to form recurrent disease. Given this, a key question is whether PSA-neg/low cells could qualify as cancer stem cells? To entertain this notion, Qin *et al.* performed a series of rigorous studies to evaluate the stemness of PSA-neg/low cells. First, PSA-neg/low cells were characterized by asymmetrical cell division (cell division to produce one daughter cell as a self-renewing copy and one copy for a differentiated lineage) and the ability to maintain cellular quiescence. The authors were able to measure asymmetrical division in LNCaP and LaPC9 cells both cell culture and xenograft studies in which lineage tracing PSA-neg/low cells could generate both PSA-neg/low and PSA-pos. cells. During clinical remission, cancer cells that survive therapy are likely quiescent with the ability to mobilize at some point to cause recurrence. Interestingly, the authors show that PSA-neg/low cells could form an increasing number of prostate spheres (an *in vitro* measure of stem cells activity) over the course of *in vitro* passaging, while maintaining low proliferation *in vivo* as measured by BrdU uptake. Second, PSA-neg/low cells expressed antigens associated with stem cells including OPN, FGF2, ALDH, integrin a2, c-Kit (CD117), CD44 and Nanog. Such observations are consistent with the authors' own previous studies[12–14] and those of others.[15] Third, a lineage hierarchy could be observed whereby PSA-neg/low cells

Department of Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA 90095, USA
Correspondence: Dr DJ Mulholland (dmulholland@mednet.ucla.edu)

were enriched for known stem/progenitor surface antigens ($ALDH^+CD44^+a2B1$), stem cell-associated transcription factors (Nanog, CD44, Nkx3.1 and OPN), but capable of differentiating to PSA-high cells. Cancer stem cell populations are often associated with drug resistance. Indeed, Qin *et al.* also demonstrated that PSA-neg/low cells expressed increased markers of stemness and drug resistance including CD44, ABCG2 and ALDH. Collectively, these data indicate that PSA-neg/low cells display qualities of cancer stem cells including the ability to self renew and differentiate to a committed lineage.

One particular interesting facet of this present report is the apparent discordance between AR and PSA expression. While most cell line analysis demonstrate a strict relationship between AR function and PSA expression, Qin *et al.* describe four populations of prostate cancer cells including AR^+/PSA^+, AR^+/PSA^-, AR^-/PSA^+ and AR^-/PSA^-. Such observations draw interesting parallels to recent studies showing that AR signaling is dispensable through compensatory PI3K/AKT signaling and that AR-low, PI3K/AKT-high regions occur in high-grade human prostate cancer.[16,17] These studies demonstrated that more effective treatment response can be achieved through the cotargeting of AR and PI3K/AKT signaling.[16,17] Thus, it will be important to determine whether PSA-neg/low prostate cancer cells observed in the present studies are also deficient for AR signaling and what other compensatory survival mechanisms are present.

Overall, the report by Qin *et al.* is important because it provides compelling evidence that advanced prostate cancer is highly heterogeneous for the AR signaling pathway. Through rigorous studies, Qin *et al.* showed that PSA-neg/low cell populations may have considerable contribution toward therapeutic resistance, progression to CRPC and metastasis. However, while the authors clearly demonstrated that PSA-neg/low prostate cancer cells do exist in primary human tumors, the molecular qualities of these cells may not necessarily mirror the characteristics obtained using PSA-neg/low *in vitro* cell lines. Thus, it will be important for future studies to develop means of isolating and differentiating the qualities of such population directly from hormone intact *vs.* CRPC as well as pre- and post-treated cell populations.

This report also underscores the fact that while the majority of prostate cancers are positive for both AR and its gene target, PSA, cells that may represent the true reservoir of CRPC and therapeutic resistance may be a population of poorly differentiated, AR-low/PSA-low cells. Could such cells represent the true villains of CRPC? If so, then perhaps future therapeutics will consider the combined use of AR inhibitors (MDV3100 and Abiraterone) with drugs having the ability to target more stem-like cells. Given recent findings that routine PSA assessments do not confer increased survival benefits, it is tempting to speculate whether a stronger correlation may exist between the content of poorly differentiated, PSA-neg/low primary tumor cells and patient survival.

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