

RESEARCH HIGHLIGHT

The complexities of identifying a cell of origin for human prostate cancer

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Prostate cancer (PCa) is the second most common malignancy in men¹ and if localized or confined to the gland at diagnosis, the choice of treatment includes surgery, radiation or watchful waiting. If and when the disease spreads, androgen blockade is effective but inevitably relapse occurs, resulting in incurable castrate-resistant PCa. Understanding the etiology of PCa will underpin the development of better treatment options. In particular, the identity of the cell type(s) that are the origin of PCa (or cancer-initiating cells) will enable them to become therapeutic targets that could lead to newer and more sophisticated treatment options. These 'cells of origin' are defined as epithelial cells in the normal prostate, susceptible to malignant transformation and therefore capable of initiating tumourigenesis. However, their identity is unknown.

In the latest issue of *Science*, Goldstein *et al.*² provide evidence that basal cells are a potential origin of human PCa, thereby substantiating their earlier report that murine basal epithelial stem cells are efficient targets for PCa initiation.³ So why are these observations notable?

The debate over the origin of human PCa has been vigorous and much has been derived from studies in mice, although unlike men, mice do not spontaneously develop PCa. A common feature of the mammalian prostate gland is the compartmentalization of the prostatic epithelium into a basal compartment, wherein there is a mixed population of cell types that includes stem cells and rapidly proliferating transit amplifying cells, and a luminal compartment, where the terminally differentiated secretory cells are located.^{4,5} These different cell types are identified and

defined by differential expression profiles for cytokeratins, androgen receptor (AR) and prostate-specific antigen (PSA) or secretory proteins.

In mice, both basal and luminal cell populations are believed to contain cancer cell(s) of origin, but in humans the tumour cells show a luminal cell phenotype and express markers that include: negative for p63 and positive for CK8/18, AR, PSA and Nkx3.1.⁶ Since the absence of p63 is used by pathologists to distinguish human tumours in specimen,⁷ a compelling case has been made to exclude the basal cell type as being a cell of origin of prostatic carcinomas, until now.

Defining and isolating human epithelial cell populations is a major obstacle to identifying a cell of origin for PCa. Commonly, intracellular markers such as cytokeratins, PSA and AR are used on tissue sections/specimens but cannot be used for cell sorting. Since intracellular and extracellular markers have not been correlated, 'no commonly accepted strategy exists to isolate such populations from dissociated human prostate tissue'.² The first sequence of data in the *Science* article by Goldstein and colleagues² shows how dispersed cells from human specimens can be sorted by fluorescence-activated cell sorting using CD49f and Trop2 to separate basal cell populations from luminal cell populations. Accordingly Goldstein *et al.*² isolated CD49f^{lo}Trop2^{hi} luminal cells (defined as CK8/18⁺CK5^{+/-}CK14^{+/-}AR⁺PSA⁺Nkx3.1⁺TMPRSS2⁺) and CD49f^{hi}Trop2^{hi} basal cells (defined as CK5⁺p63⁺CK14^{+/-}CK8/18^{+/-}). Although they did not comment on this, the populations are heterogeneous and in particular the basal cell populations will also contain intermediate (transit amplifying) cells. It remains to be shown, using their sorting strategy, that specific cells are tumourigenic as it would be expected that only a rare subpopulation

will have this property. Maitland and his colleagues have already used CD133⁺/α2β1 integrin/CD44⁺ to enrich for a cancer-initiating stem/progenitor cell population from human PCa,⁸ and improving the purity of the cells of origin for PCa, will be critical to the success of therapeutic targeting.

Following on from the development of a suitable protocol, Goldstein *et al.* show that CD49f^{hi}Trop2^{hi} basal cells generated fully differentiated glandular ducts, whereas CD49f^{lo}Trop2^{hi} luminal cells failed to give rise to epithelial structures when grafted with inductive stroma (urogenital sinus mesenchyme) in NOD scid gamma (NSG) mice. These data were less surprising, since normal stem cells are postulated to reside in the basal and/or intermediate epithelial compartments of benign prostate.⁹

They also report how they elegantly inserted mutations into the basal or luminal cell populations and that the basal cells bearing these mutations generate carcinomas, but the luminal cell populations did not. Subfractionated cells were transduced with lentivirus, carrying both red fluorescent protein-tagged activated (myristoylated) AKT and ERG into basal (CD49f^{hi}Trop2^{hi}) or luminal (CD49f^{lo}Trop2^{hi}) cell populations. Abnormal structures, characteristic of high-grade prostatic intraepithelial neoplasia (PIN), arose from the basal (but not luminal) cell populations. Further genetic alteration, including green fluorescent protein-tagged AR lentivirus, showed that the combination of AKT, ERG and AR resulted in the development of adenocarcinoma (and/or PIN) from basal cells, but not luminal cells. The conclusion that a basal cell population is a cell of origin for PCa is therefore justified.

The failure of the luminal cell populations to generate PIN or malignant tissue could be interpreted as evidence that these cell populations are not cancer-initiating cells.

However, the luminal (CD49^{lo}Trop2^{hi}) cell populations failed to survive in the transplant system and therefore, their tumor-forming capacity could not be formally tested in the subsequent studies of tumorigenicity. The take rates of xenografts of low grade PCa or benign specimens is notoriously poor and has been a major roadblock in research activity for many years,¹⁰ whereas high-grade PCa specimens are relatively easy to grow. Thus, the failure of the (CD49^{lo}Trop2^{hi}) luminal cell populations to generate PIN or malignant tissue is likely to be due to the limitations of the grafting procedure itself and cannot be interpreted as a failure of this population of cells to be the origin for PCa, without further proof. The idea that luminal cell populations are cancer-initiating cells was recently reported by Shen *et al.*,¹¹ who described a rare epithelial luminal cell population, which is castrate resistant and expresses the Nkx3.1 homeobox gene (CARN cells), as a potent cell of origin for murine PCa. Presumably this, or the equivalent, subpopulation of cells could be present in the human CD49^{lo}Trop2^{hi} luminal cells and remains to be tested for tumorigenicity.

These latest discoveries are a major advance for the field, being the first to use human prostate specimens to address this critical area of research. Identification of cancer-initiating cell populations will allow the field to focus on how to target these specific cells and develop new therapies. The report is notable because it demonstrates that the basal cell populations contain cells that, when mutated, are cancer-initiating.² However, in all probability it is likely that there will be more than one cancer cell of origin (as has been demonstrated in breast cancer),¹² and the PCa field will have to consider both luminal and basal cell types in its approaches to the discovery of new therapies for PCa.

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