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RESEARCH HIGHLIGHT

Human prostate cancer stem cells: new features unveiled

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Cancer stem cells (CSCs) are a rare subpopulation of phenotypically distinct cancer cells exhibiting stem cell characteristics. They are tumourigenic, meanwhile capable of self-renewal and forming differentiated progenies. CSCs are believed to be resistant to the standard therapeutics, and provide the cell reservoir for tumour initiation.[1] Understanding CSCs or in another word, tumour-initiating cells, is of critical therapeutic importance.

Prostate cancer is the most commonly diagnosed cancer in males in the Western world. The search for markers of CSCs in human prostate cancer has been ongoing for years. Studies from a few labs successfully isolated a subpopulation of cells that had enhanced tumourigenicity *in vitro* and/or *in vivo*, using cells from either established prostate cancer cell lines or xenografts. Examples of markers for such subpopulations include $CD44^+$, $CD133^+$, Integrin $\alpha2\beta1^{hi}$, β-catenin$^+$, etc (summarized in Ref. 2). Recently, using genetically engineered basal cells (marked by $CD49f^{hi}Trop2^+$) from human prostate specimen, Goldstein *et al* successfully reconstituted prostate cancer in immunodeficient mice.[3] However, many of these markers represent a substantial percentage of the total population, and definitive markers for prostate cancer stem cells are still lacking. In a recent issue of *Nature Communications*, a study by Rajasekhar *et al.* significantly expanded our current understanding of prostate CSCs, and identified two novel features of CSCs in prostate cancer: (i) expression of TRA-1-60, CD151 and CD166; (ii) elevated NF-κB signalling.[4]

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First of all, the authors successfully enriched stem-like prostate cancer cells from a xenograft model using sphere culture, an *in vitro* surrogate for accessing 'stemness'.[4] A small percentage of primary cells isolated from the orthotopic CWR22 tumours formed spheroids. These spheroids exhibited stem-like properties. They were multipotent, with at least 100 times higher tumour-initiating capacity compared to the overall population. They were dedifferentiated, as evidenced by no androgen receptor or PSA expression.[4] It is noteworthy to mention the controversy about whether the basal cell or luminal cell subpopulation is the cell of origin for prostate cancer.[5] Wang *et al.* used a mouse model and showed that PTEN deletion in CARNs that represented a rare population in luminal cells gave rise to prostate cancer.[6] In contrast, Witte's group used FACS-sorted primary cells and showed that basal cells but not luminal cells were the cell of origin for prostate cancer.[3,7] Interestingly, the CSCs identified here by Rajasekhar *et al.* were basal-like but with certain attributes of intermediate/luminal cells as well. These cells overall looked basal since they were negative to AR, PSA, Nkx3.1, CK18 and positive to CK5, but lacked an crucial basal cell marker p63 and expressed at least one luminal cell marker CK8.[4]

Secondly, the authors sought to identify cell surface markers that would facilitate the direct purification of the stem-like prostate cancer cells.[4] Consistent with the literature, cells expressing some traditional prostate stem cells markers such as CD44 and CD133 grew more spheres. However, the authors decided to go a step further and searched for novel makers that would better define the prostate CSCs. It turned out that TRP-60-1, particularly when coexpressed with CD151 and CD160, significantly enriched the prostate CSCs. Importantly, TRP-60-1^+/$CD151^+$/$CD160^+$ cells had significantly higher capacity of *in vitro* sphere formation and *in vivo* tumour generation, and were capable of both self-renewal and differentiation by recapitulating a cellular hierarchy of the original parental tumour.[4] Androgen deprivation therapy is a standard of care for prostate cancer. A major clinical hurdle is the castration-resistant population that survives androgen deprivation, and will repopulate and emerge as castration-resistant tumours. It would be desirable to know if the TRP-60-1^+/$CD151^+$/$CD160^+$ cells are truly the driving force of castration-resistant tumours in future experiments.

Thirdly, having demonstrated TRP-60-1^+/$CD151^+$/$CD160^+$ as a novel feature for prostate CSCs in CWR22 orthotopic xenograft model,[4] the authors decided to determine if this observation was restricted to the one prostate cancer model used. In other words, is it a universal identity for prostate CSCs? The authors examined several other representative prostate cancer models including DU-145, PC3, VCaP and PC-82 implanted either subcutaneously or orthotopically. Results from all prostate cancer models tested consistently showed that the triple positive cells represented a rare population (0.1%–0.5%) and exhibited elevated tumour-initiating capacities that sustained serial passages. Moreover, such triple positive cells were also detected as a rare population (~2.5%) in human prostate clinical tumours from radical prostatectomy, and were detected at a higher percentage in prostate tumours than the matching non-tumours.[4] It is still unknown whether or not such triple positive cells isolated from human clinical prostate tumours could show enriched tumour-formation capability *in vivo*.

Next, in order to provide some mechanistic insights, the authors further

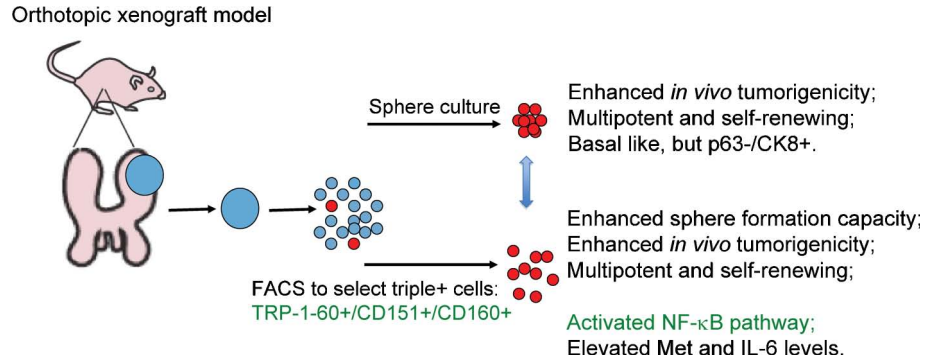

Figure 1 Identification of prostate cancer stem cells (CSCs). Human prostate cancer cells were isolated from CWR22 tumours grown orthotopically in mice. Prostate CSCs were enriched through either sphere cultures or FACS. Two new features of prostate CSCs were identified: (i) expression of TRP-1-60$^+$/CD151$^+$/CD160$^+$; (ii) activation of NF-κB signalling.

explored the gene expression profiles and signalling status of the TRP-60-1$^+$/CD151$^+$/CD160$^+$ prostate CSCs.[4] A list of molecules including microRNAs displayed differentiated expression/activity between CSCs and non-CSCs, which included pro-inflammatory cytokine IL-6, NF-κB signalling, Met, PKCα phosphorylation and anti-apoptotic Bcl-2 family member MCL-1. The elevated level of IL-6 is consistent with a recent study showing that IL-6 could convert non-stem cancer cells to CSCs, in both mammary and prostate cancer models.[8] The constitutively activated NF-κB signalling in the prostate CSCs is reminiscent of some recent studies. Ammirante *et al.* showed that NF-κB signalling was critical for lymphotoxin-protected prostate cancer cell survival.[9] Also, in consideration of the association between CSCs and tumour metastasis, it was conceivable that NF-κB modulated the metastasis of prostate cancer.[10] On the other hand, it is a little bit surprising that none of the previously identified prostate CSC markers (e.g., CD44 or CD133) were elevated in the triple positive cells, which might be potentially explained by a possible disconnection between mRNA levels and protein levels. Alternatively, it is also possible that the TRP-60-1$^+$/CD151$^+$/CD160$^+$ cells may represent a distinct population from the prostate CSCs previously identified.

Lastly, a better understanding of the signalling status of the prostate CSCs may provide potential therapeutic targets. Remarkably, small molecule inhibitors that target NF-κB signalling or Met successfully blocked secondary sphere-formation *in vitro* and tumour-initiation *in vivo*.[4] Even though no data were provided about whether these inhibitors actually eliminated TRP-60-1$^+$/CD151$^+$/CD160$^+$ cells, it does sound very promising as shown by a different study that silencing IKKα could delay the emergence of castration resistant prostate cancer.[9] More studies are needed to directly address whether eliminating TRP-60-1$^+$/CD151$^+$/CD160$^+$ CSCs would diminish or delay the occurrence of castration resistant prostate cancer.

In summary, the study by Rajasekhar *et al.* has revealed a pivotal feature for prostate CSCs: TRA-1-60$^+$/CD151$^+$/CD166$^+$ (**Figure 1**). The constitutive activation of NF-κB signalling in the identified CSCs implicates blocking NF-κB signalling as possible therapeutic strategy for eliminating/inhibiting prostate CSCs. The fact that the CSCs identified are basal-like but express luminal marker CK8 as well adds more layer to the discussion of cell of origin for prostate cancer. The important discoveries in this study lead to several apparent questions: (i) Would the TRP-60-1$^+$/CD151$^+$/CD160$^+$ cells isolated from human prostate clinical tumours capable of reconstituting tumours *in vivo*?; (ii) What is the relevance of the TRP-60-1$^+$/CD151$^+$/CD160$^+$ cells in the development of castration resistant prostate cancer?; (iii) Would eliminating TRP-60-1$^+$/CD151$^+$/CD160$^+$ cells block the occurrence of castration resistant prostate cancer?

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