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Detection of Potential Epithelial Mesenchymal Transition Cells in Localized Prostate Cancer

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Introduction

Circulating tumor cells (CTCs) are well characterized in advanced metastatic prostate cancer, however CTC detection and possible clinical utility in non-metastatic, localized prostate cancer (LocPca) is controversial (Maas et al.). A primary criticism of early studies evaluating CTCs in LocPca is that the majority of CTC detection was conducted via the CellSearch platform which selectively captures CTCs with an epithelial phenotype (EPCAM, CK+). Recent studies have emphasized the importance of non-traditional CTCs, such as epithelial mesenchymal transition cells in prostate as well as other cancers (ref). The Target Selector™ CTC platform can also detect cytokeratin negative (CK-) cells which are potential EMT transitional cells. We report on the rate of both traditional (CK+) and non-traditional CTCs (CK-) cells in LocPca.

Methods

CTC results from deidentified clinical data was analyzed. Patients were diagnosed with non-metastatic disease and either were placed on active surveillance or had first line therapy (surgery or EBRT). (N=75) The capture antibody mixture contained anti-EpCAM (Trop-1), tumor-associated calcium signal transducer 2 (Trop-2), anti-c-MET, anti-Folate-binding protein receptor, anti-N-Cadherin, anti-CD318, and anti-mesenchymal stem cell antigen. Cells were stained with a mixture of anti-cytokeratin 17, 18, 19, pan-cytokeratin, CD45 antibody labeled with AlexaFluor-594 and DAPI III to visualize the nucleus. CTCs were captured and detected on a microchannel platform derivatized with streptavidin and mathematically designed to avoid laminar flow to maximize cell contact. CTC enumeration was performed via Olympus BX51 fluorescence microscope at 200 X magnifications and based on CK+/CD45-/DAPI+ stain criteria. The precise location of each CTC was recorded, permitting cell re-localization.

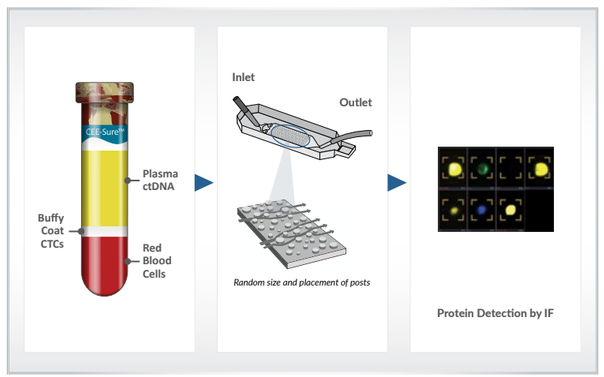

Figure 1. Biocept platform for CTC capture and staining. CTCs are captured in transparent microfluidic channels and can be viewed in situ by fluorescent microscopy. CTCs can be analyzed via immunofluorescence (IF)

Results

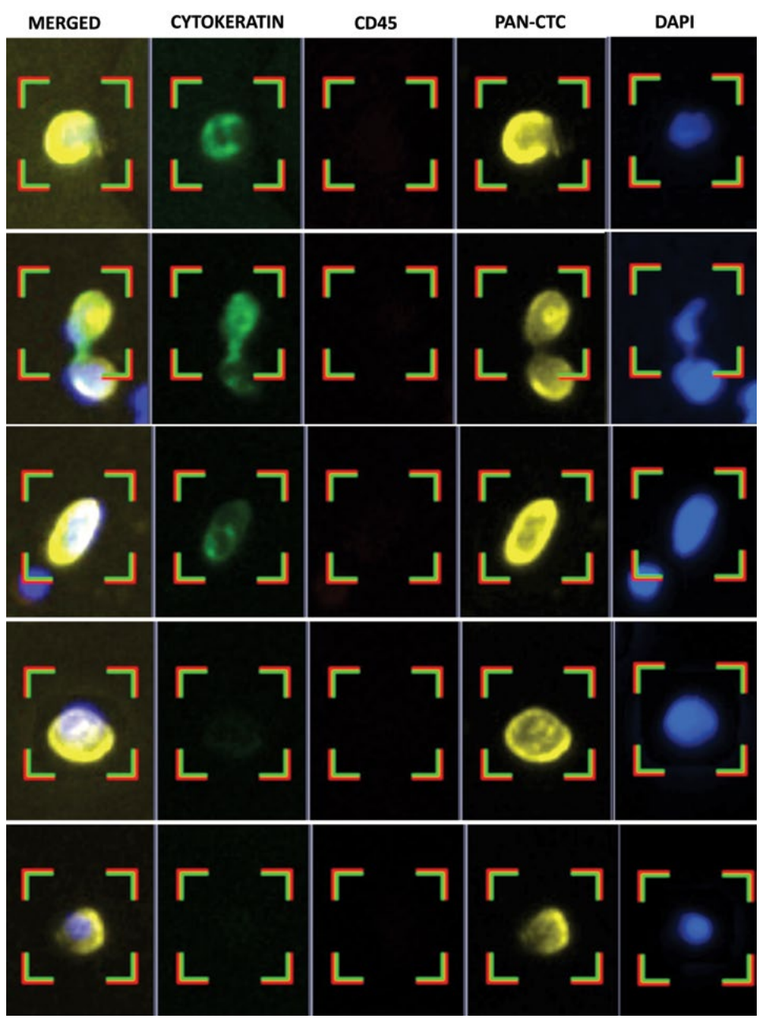

Figure 2. Detection of epithelial and stem-like CTCs enriched from prostate patient blood. CTCs are identified with the pan-CTC stain. The first 3 cells in this example stain for the epithelial cytokeratin marker, while last 2 cells, which are more stem like do not stain for cytokeratin. All CTCs are CD45 negative.

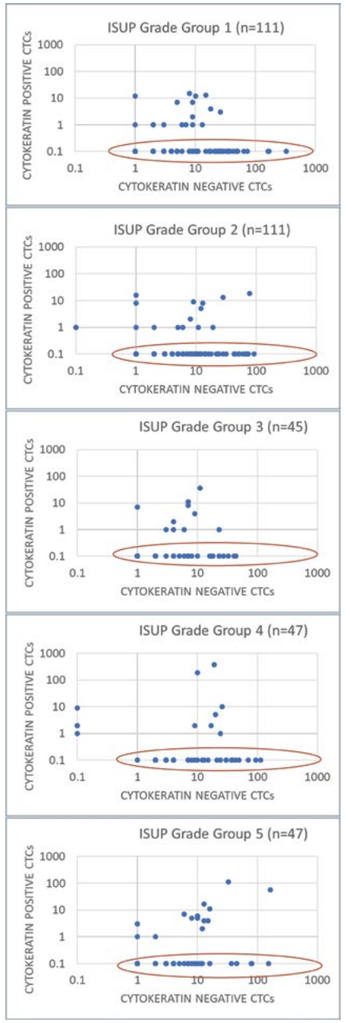

Figure 3. High detection rate of cytokeratin negative CTCs (pan-CTC stain positive CTCs_) in all prostate cancer grade groups. Each data point (dot) represent number of CTCs detected in a blood sample from a prostate tumor patient. The red circle indicates patients with only cytokeratin negative CTCs.

Results

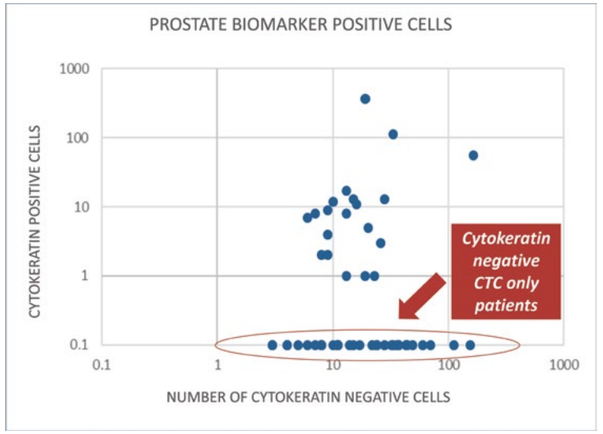

Figure 4. Cytokeratin negative CTCs are positive for tumor biomarkers. The red circle indicates patient samples with only cytokeratin negative CTCs. Each dot represents a patient sample and indicates number of cytokeratin positive vs cytokeratin negative tumor cells detected. This plot includes only patient samples positive for one or more tumor markers (EGFR amplification, ARv-7 protein, PTEN deletion, c-met amplification, c-myc amplification)

Conclusions

These results demonstrate that cytokeratin negative CTCs are detected more frequently than cytokeratin positive CTCs and represent a population that are currently not captured and considered due to the epithelial CTC focus of the leading CTC detection platform. Ongoing research will better characterize these cells as potential EMT phenotype and their association with clinical parameters.

References

1. Mikolajczyk SD, Millar LS, Tsinberg P, et al. Detection of EpCAM-Negative and Cytokeratin-Negative Circulating Tumor Cells in Peripheral Blood. J Oncol. 2011;2011:252361.