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EMT-Like Circulating Tumor Cells (CTCs) in the Blood of Early-To-Late Stage Prostate Cancer Patients

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ABSTRACT

Microfiltration is an increasingly popular method for isolating circulating tumor cells (CTCs) from the peripheral blood of cancer patients with solid tumors. This size exclusion method does not rely on surface marker expression of CTCs, making it ideally suited for isolating specialized cancer cells that have undergone epithelial-mesenchymal transition (EMT). We have found a specific subtype of CTCs in both early and late stage prostate cancer patients that is indicative of the EMT phenotype, presenting with low cytokeratin and low or no EpCAM. Further detailed molecular analysis and patient tracking of this phenotype may aid in individualized assessment of early clinical assessment of early stage cancer patients.

MATERIALS & METHODS

Blood samples from prostate cancer patients (stage I-IV) were provided by Northwestern University and Fox Chase Cancer Center. Microfilters were fabricated with 7 micron diameter pores in a uniform array over a 9 mm diameter area. 7.5 mL of whole blood was diluted 1:1 in a fixative and filtered through CellSieve™ microfilters (~3 min). CTCs collected were then fixed, permeabilized, and stained with DAPI and antibodies specific to CK 8, 18 and 19 (FITC), EpCAM (PE), and CD45 (Cy5). Cells without CD45 staining were classified by their morphology, nuclear integrity and the presence of cytokeratin and EpCAM. A specific subgroup of CTCs with low cytokeratin, low or no EpCAM and CD45 negative were identified in most patient samples.

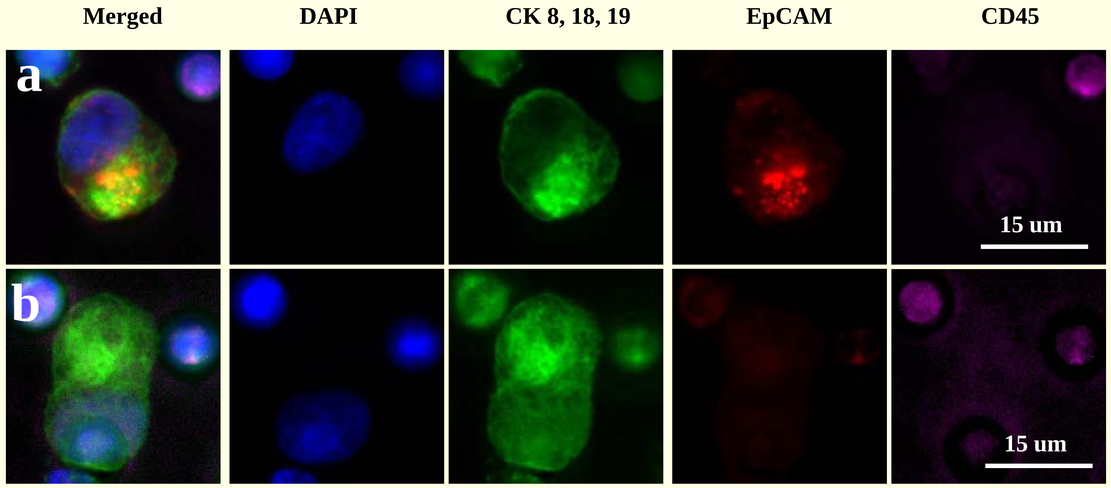

Figure 1. Traditional CTCs. (a) CTC with high cytokeratin filamentation and high EpCAM expression. (b) CTC with high cytokeratin expression and no EpCAM expression

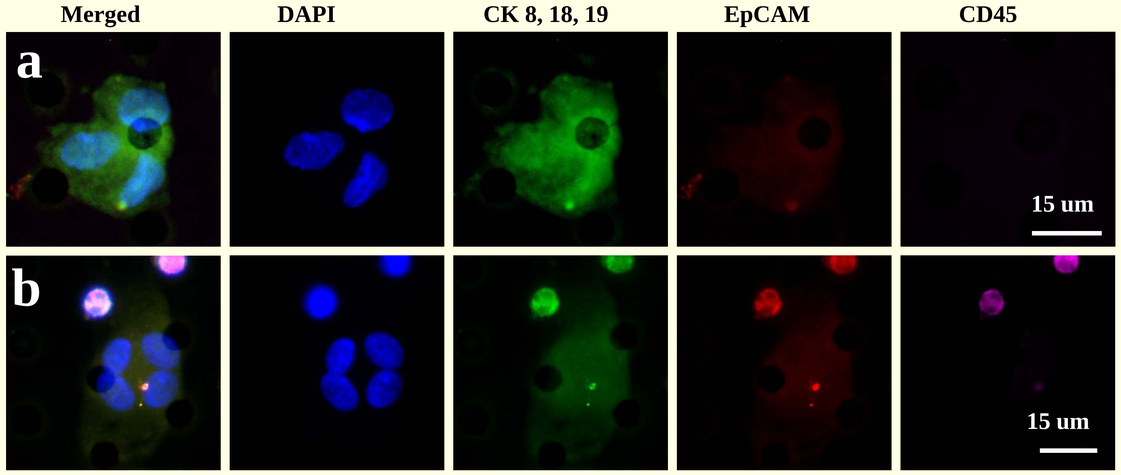

Figure 2. Clusters of EMT-Like CTCs. (a) Cluster of CTCs with 3 nuclei (b) Cluster of EMT-like CTCs with 4 nuclei.

INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. CTCs can be used to monitor therapy response and predict outcome.[1-4] CellSieve™ microfilters are lithographically fabricated membranes with high porosity, precise pore dimensions, and patterned pore distribution. We previously reported that CellSieve™ rapidly and efficiently isolates CTCs from whole peripheral blood, using fluorescent antibody stain as the detection platform.

It has been shown that a specific subset of CTCs undergo differentiation into a migratory phenotype with high metastatic potential, termed epithelial-mesenchymal transition (EMT). These EMT CTCs lose their typical surface markers and express more mesenchymal phenotypes. As size exclusion is irrespective of surface marker expression, this approach may be optimal for isolating EMT CTCs.[2-4]

RESULTS

- Assay time was <2 hours.
- Traditional CTCs were easily identified by fluorescent stains in 25% of samples (Figs. 1a-b).
- EMT-like CTCs were identified by their abnormal nuclear pattern in 63% of samples (Figs. 2-3).
- EMT-like CTCs were found to have low cytokeratin expression and often lack EpCAM (Figs. 2-3).
- EMT-like CTCs were often found in large multi-cell clusters (Figs. 2a-b and 3b).
- EMT-like CTCs were consistently found in all cancer stages.

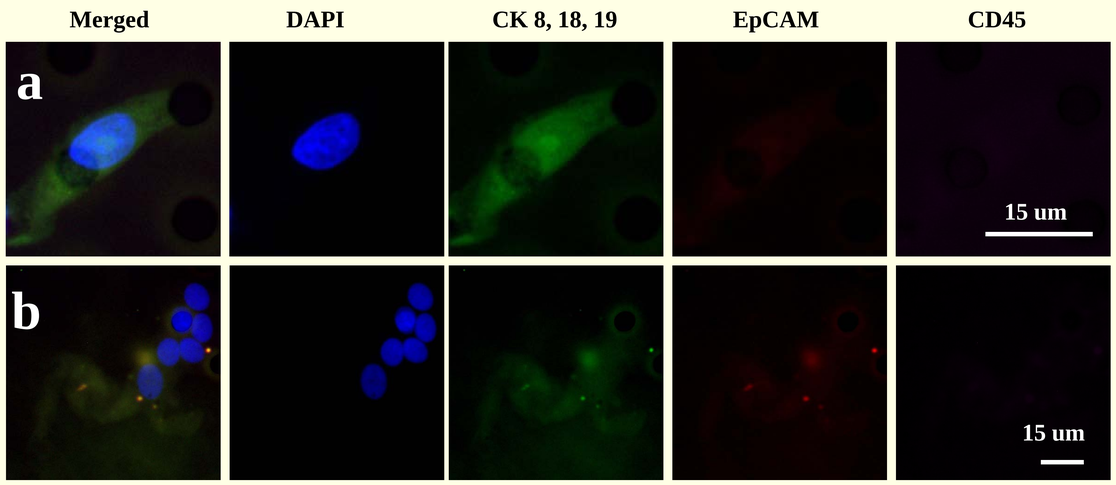

Figure 3. Spindled structured EMT-like CTCs. (a) Single spindle shaped EMT-like CTC. (b) Cluster of EMT-like CTCs in a spindle shape.

CONCLUSIONS

- Microfiltration captures CTCs regardless of EpCAM expression.
- EMT-like CTCs circulate in the blood of prostate patients.
- EMT-like CTCs are found in stage I – stage IV patients.
- Further longitudinal study of EMT-like CTCs is expected to provide additional information about early patient assessment.

References

1. Pachmann, K., *et al.* (2008). "Monitoring the response of Circulating Epithelial Tumor Cells to Adjuvant Chemotherapy in Breast Cancer Allows Detection of Patients at Risk of Early Relapse." J. of Clin. Oncol. 28 (8):1208-1215
2. Vona, G, *et al.* (2000). "Isolation by Size of Epithelial Tumor Cells A New Method for the Immunomorphological and Molecular Characterization of Circulating Tumor Cells." American Journal of Pathology 156(1): 57-63.
3. Lecharpentier, *et al.* (2011). "Detection of circulating tumour cells with hybrid (epithelial/mesenchymal) phenotype in patients with metastatic non-small cell lung carcinoma." BJC. 105:1338-1341
4. Yu, M, *et al*. (2013). "Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition." Science 339:580-584