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Identifying and Subtyping Circulating Tumor Cells (CTCs) from breast, prostate and pancreatic cancer patients based on distinct morphology

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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[2-4]. The microfiltration approach can be used on peripheral blood as a non-invasive "liquid biopsy" for precision cancer detection, regardless of surface marker expression[2-4]. Here we describe the use of CellSieve™ microfilters to isolate and subtype CTCs from the peripheral blood of breast, prostate and pancreatic cancer patients. As it is accepted that CTCs isolated from patient samples represent a highly heterogeneous population with varying degrees of epithelial/mesenchymal differentiation, microfilter isolation may be optimal for the purification of all CTC subtypes. We hypothesize that CTCs from three different epithelial malignancies can be identified and grouped into distinct subtypes by morphological characterization.

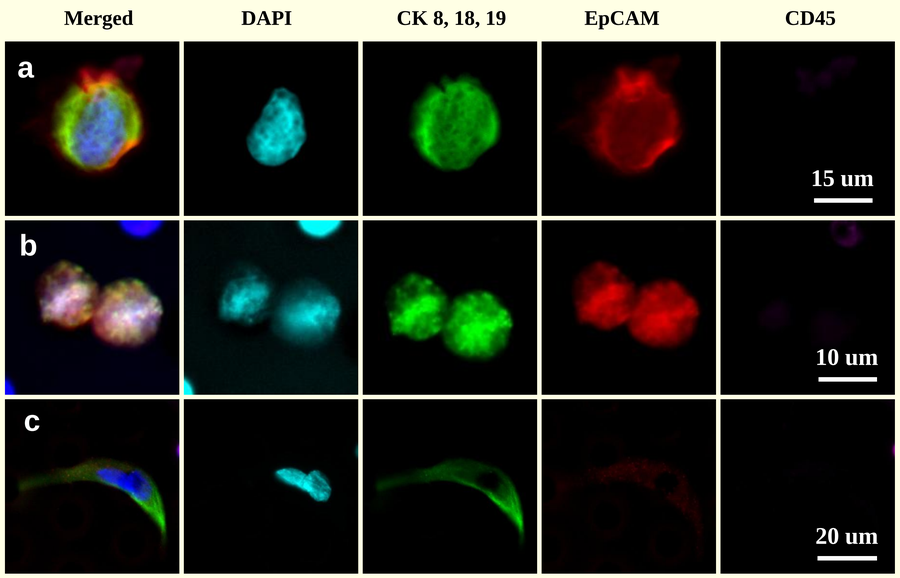

Figure 1. Morphological variations of CTCs
- **a) Breast CTC with high EpCAM expression and filamentous cytokeratin**
- **b) Prostate CTC with high EpCAM and mottled cytokeratin**
- **c) Pancreatic CTC with fine filamentous cytokeratin and low EpCAM**

INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. It has been well established that CTC enumeration can be used to monitor therapy response and predict outcome.[1-4] Size exclusion is a technique for isolating CTCs from patient samples, irrespective of their surface marker expression.[2-4]

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. In addition to enumerating CTCs, subtyping by phenotypic determinates may aid in identifying the CTCs cellular status for diagnosis, prognosis and therapy determination.[1-4]

MATERIALS & METHODS

Blood from breast, prostate, and pancreas cancer patients were provided by Northwestern University, Fox Chase Cancer Center, University Maryland Greenebaum Cancer Center, and Medical College of Wisconsin, and analyzed by Creatv MicroTech. Microfilters are fabricated with 7 micron diameter pores and uniform array of160,000 pores over a 9 mm diameter area. 7.5 mL of whole blood was mildly pre-fixed and filtered through CellSieve™ microfilters (~3 min). CTCs collected were then fixed, permeabilized, and stained with DAPI, and antibodies to cytokeratin (CK) 8, 18 and 19 (FITC), EpCAM (PE), PSMA (Dylight 594) and CD45 (DyLight 649). CTCs were classified by their morphology, nuclear profile and the expression patterns of cytokeratin, PSMA and EpCAM.

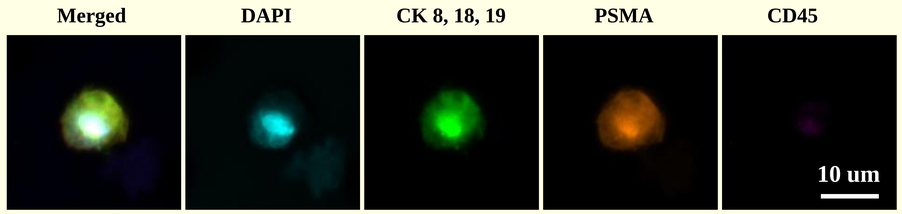

Figure 2. CTCs stained with a additional PSMA antibody

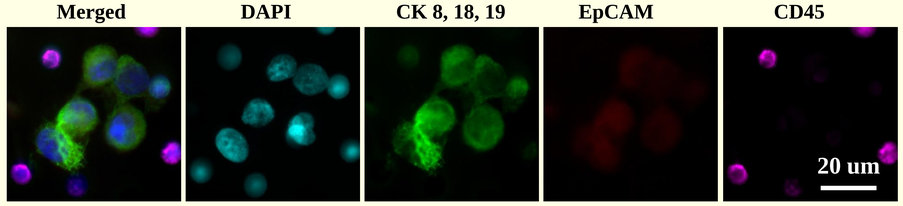

Figure 3. Cluster of CTCs with filamentous cytokeratin from a breast cancer patient with low expressing EpCAM

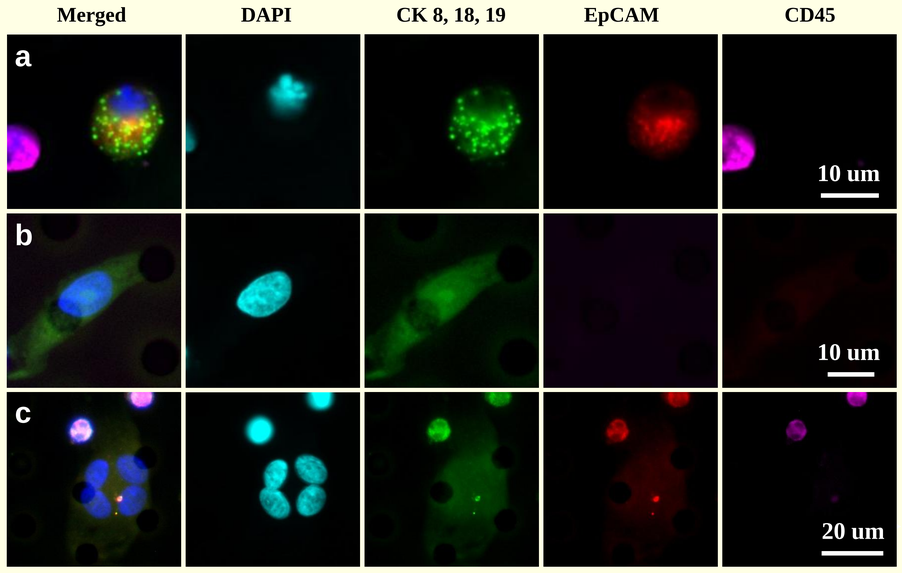

Figure 4. Subtypes of CTCs within cancer families (a) Prostate, early apoptotic, (b) Prostate, EMT-Like CTC (c) Prostate, EMT-like cluster of CTCs

RESULTS

- The three malignancies have distinct identifiable morphologies
  - Breast – high CK expression in a filamentous pattern (Figs. 1a and 3).
  - Prostate – high PSMA and CK expression in a mottled pattern (Fig 1b and 2)
  - Pancreatic – CK expression with a fine filamentous pattern and spindle-like cellular structure (Fig. 1c)
- Within each cancer CTCs could be subdivided
  - EMT-like CTCs – low expressing CK with smooth nuclear profile (Fig 4b and 4c)
  - Apoptotic CTC – spotted nuclear and CK patterns (Fig 4a)
  - CTC Clusters – CTCs found in clusters (Fig 1b, 3, & 4c)
- PSMA can verify identified CTCs as prostate cells (Fig. 2).
- Phenotypic traits can be identified and classified for comparative analysis (Figs. 1-3).

CONCLUSIONS

- Microfiltration captures CTCs regardless of surface marker expression
- CTCs have multiple distinct phenotypes
- CTC phenotypes differ between malignant diseases
- Microfiltration captures weakened and apoptotic CTCs.
- CTC subtypes may indicate definable traits which may be exploited for personalized treatment of cancer patients

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