

Morphologic Variation of Circulating Tumor Cells (CTCs) from Prostate Cancer Patients Isolated using Precision Microfilters

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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 tumors²⁻⁴. The microfiltration approach can be used on peripheral blood as a non-invasive liquid biopsy for precision cancer detection and subtyping²⁻⁴. Here we present a use of the CellSieve™ microfilter to subtype CTCs based on the immunofluorescent staining pattern of cytokeratin filamentation, PSMA presence and EpCAM surface marker expression.

Our initial studies on CTCs in patient blood indicates that disseminated CTC populations have high rates of phenotypic heterogeneity, though specific patterns exist within cancers. Further detailed molecular analysis and patient tracking of these phenotypes may lead to individualized patient assessment based on CTC characterization.

MATERIALS & METHODS

Blood samples from metastatic prostate cancer patients 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), PSMA (Dylight594) and CD45 (DyLight 649). Cells without CD45 staining were classified by their morphology, nuclear integrity and the presence of cytokeratin, PSMA and EpCAM.

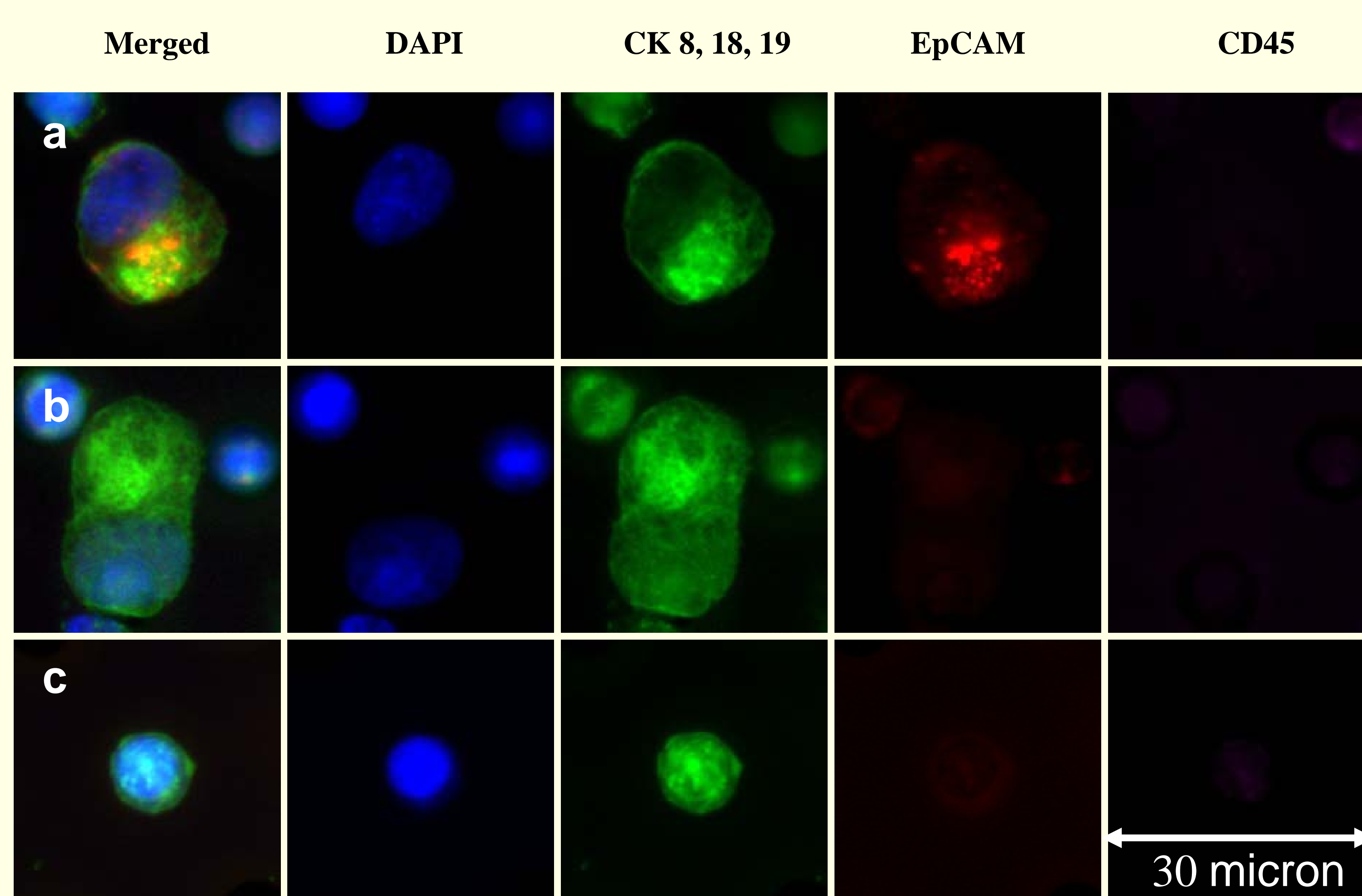


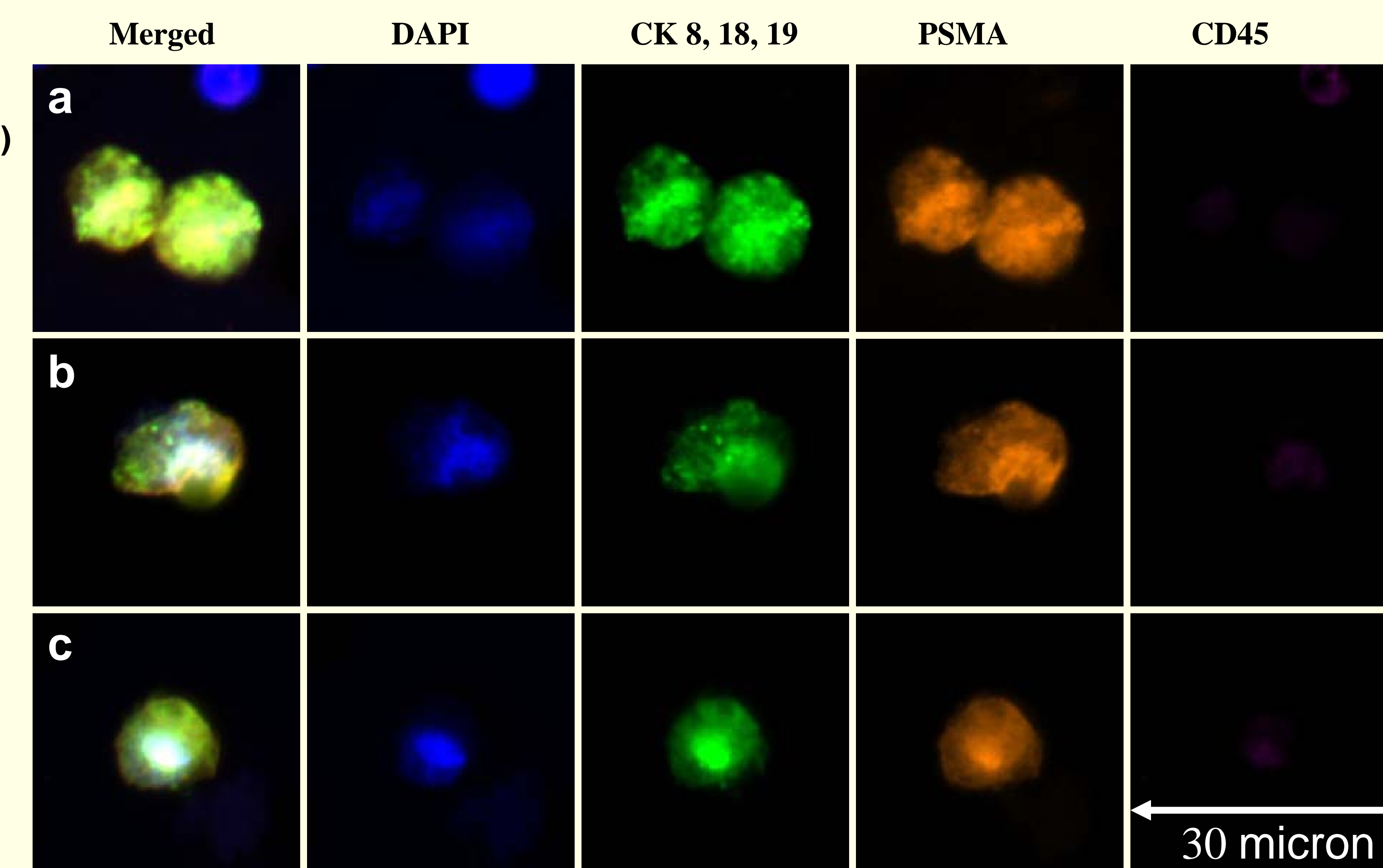
Figure 1. Morphological variations of CTCs

- (a) CTC with high EpCAM expression and cytokeratin filamentation
(b) Filamented CTC with no EpCAM
(c) Small CTC with no EpCAM

- Figure 3. CTCs stained with a additional PSMA antibody**
(a) CTC pair (micrometastasis)
(b) Early apoptotic CTC with PSMA
(c) Single PSMA positive CTC

RESULTS

- Assay time was <2 hours.
- CTCs were easily identified by traditional fluorescent stains (Figs. 1a-c and 3a-c).
- CTCs had high, low, or no EpCAM expression (Figs. 1-3).
- CTCs had variable Cytokeratin expression and patterns (Figs. 1-3).
- PSMA identified CTCs as prostate cells (Fig. 3).
- Apoptotic CTCs were identified by a spotted morphology (Figs. 2a & b).
- Cell clusters, PSMA, and other phenotypic traits could be identified and classified for comparative analysis (Figs. 1-3).
- CTC morphology varied by stage & therapy (Figs. 1-3).



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 Size exclusion is a technique for isolating CTCs 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. Further subtyping by phenotypic determinates may aid identifying the CTCs cellular status for diagnosis, prognosis and therapy determination.¹⁻⁴

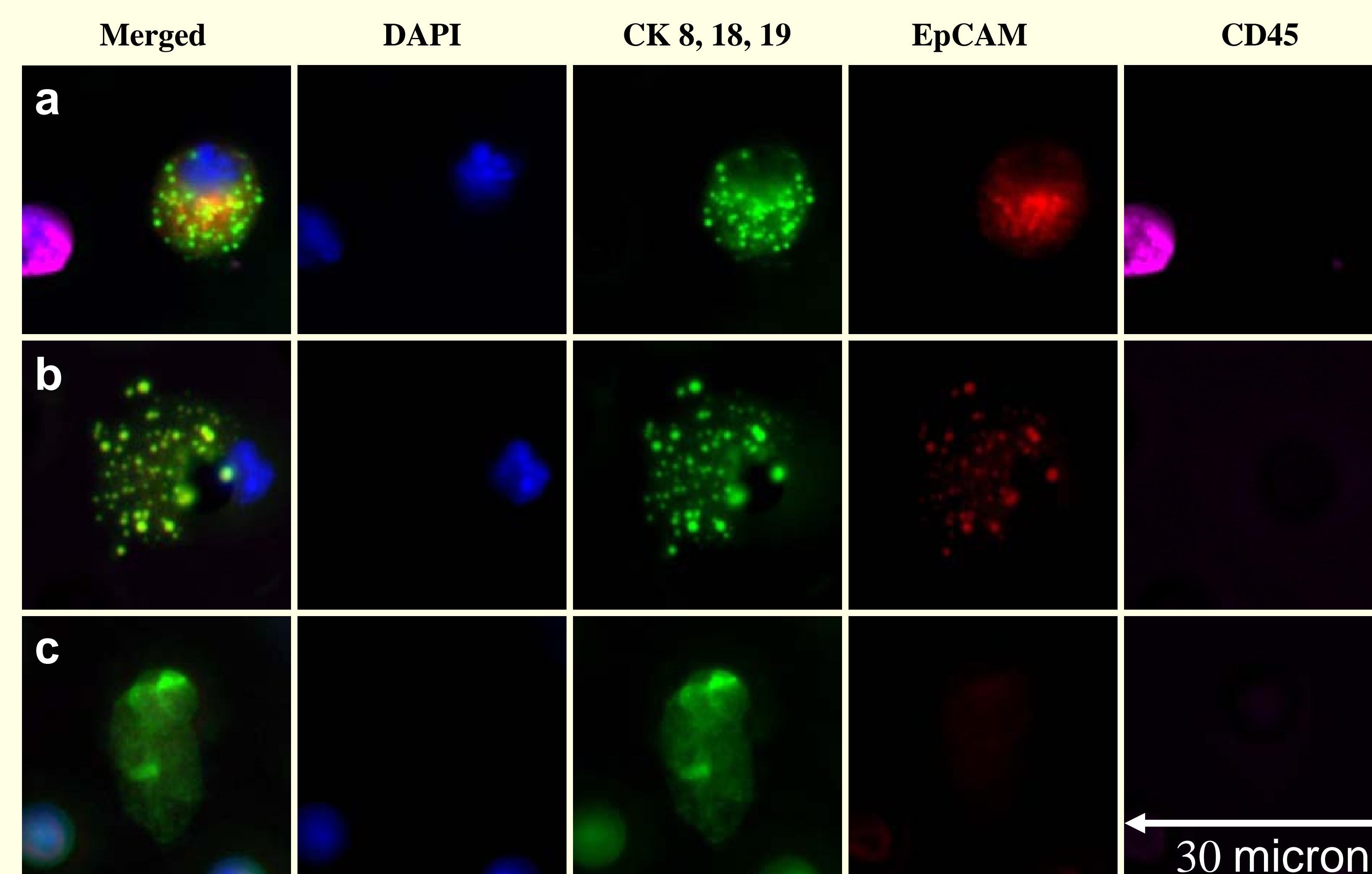


Figure 2. Apoptotic CTCs (a) Early apoptotic, (b) Late apoptotic, (c) Denucleated CTC

CONCLUSIONS

- Microfiltration captures CTCs regardless of EpCAM expression.
- Microfiltration captures weakened and apoptotic CTCs.
- CTC phenotypes differ according to treatment and disease stage.
- Further longitudinal study of patient CTCs is expected to provide additional information about patient assessment.

References

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