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Low Cytokeratin- and Low EpCAM-Expressing Circulating Tumor Cells in Pancreatic Cancer

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ABSTRACT

To date, circulating tumor cells (CTCs) in the peripheral blood of pancreatic patients using standard immuno-capture techniques has met with limited success. As pancreatic cancer is prone to metastasize at distant sites, and therefore should have high number of CTCs, it is possible that immuno-capture methods are not suitable for this disease. Microfiltration is an increasingly popular method for isolating CTCs from the peripheral blood of cancer patients with solid tumors, regardless of surface marker expression[2-4]. Using a microfiltration approach, we show that two distinct CTC subtypes can be identified in the peripheral blood in 80% of pancreatic patients.

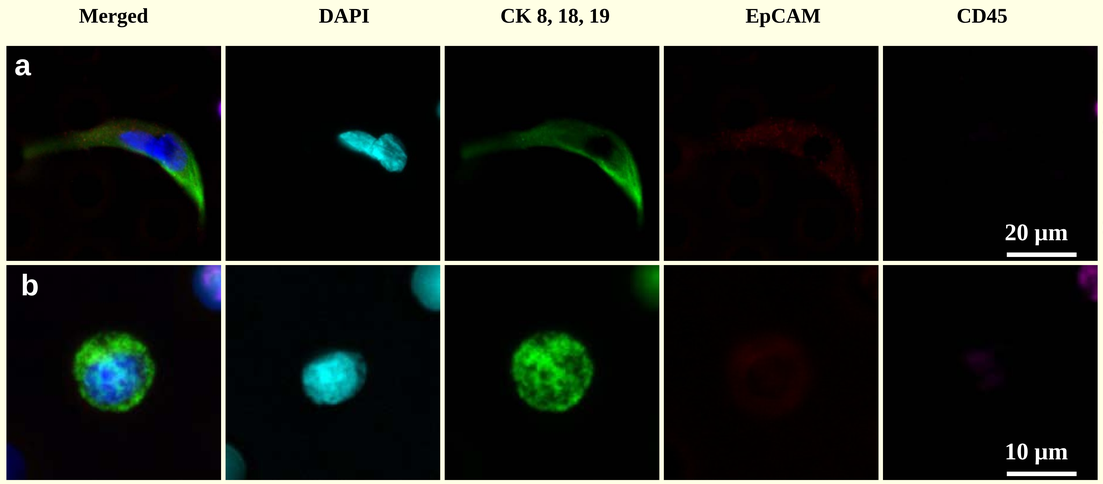


Figure 1. Pathologically defined CTCs
(a) Pancreatic CTC with low EpCAM expression and elongated filamentous cytokeratin
(b) Pancreatic CTC with filamentous cytokeratin and no EpCAM

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]

Figure 2. EMT-Like CTCs
(a) Pancreatic CTC with high EpCAM expression and weak cytokeratin staining
(b) Pancreatic CTCs with low EpCAM and weak cytokeratin staining

MATERIALS & METHODS

Pancreatic patient samples (n=50) were provided by Medical College of Wisconsin, Milwaukee, WI. CellSieve™ microfilters, with precision 7 micron diameter pores distributed in uniform arrays were employed. 7.5 mL of whole blood was diluted in pre-fixation solution and filtered through CellSieve™ microfilters (~3 min). CTCs collected were fixed, permeabilized, and stained with DAPI, and an antibody cocktail against cytokeratin (CK) 8, 18 and 19 (FITC), EpCAM (PE), and CD45 (Cy5). CTCs, defined as cytokeratin positive and CD45 negative, were found in two distinct subtypes. One subtype had the “classic” characteristics of a CTC, with high EpCAM and cytokeratin expression, identifiable filamentation, and a cancer-like nuclear structure. The second subtype is indicative of a CTC undergoing epithelial-mesenchymal transition (EMT), with low or no EpCAM, weak cytokeratin expression, and a smooth oval nuclear structure.

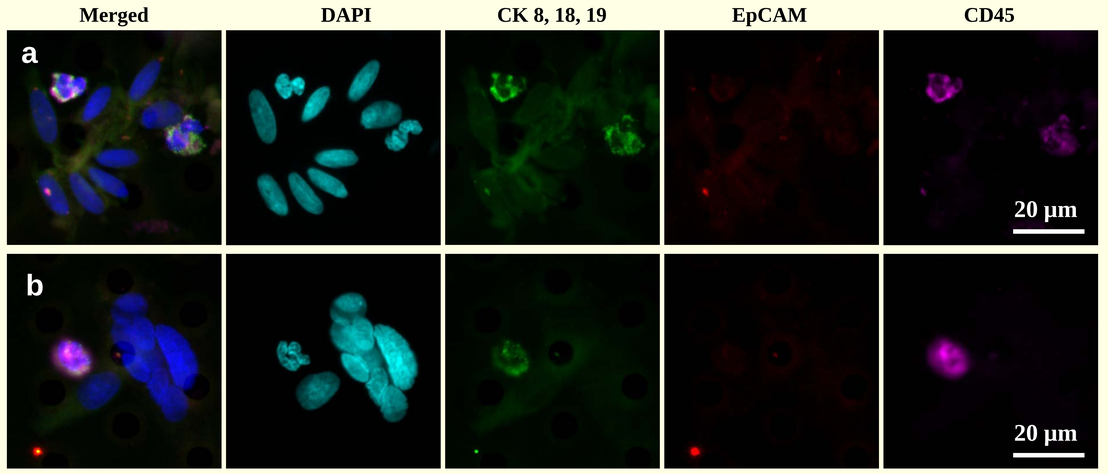
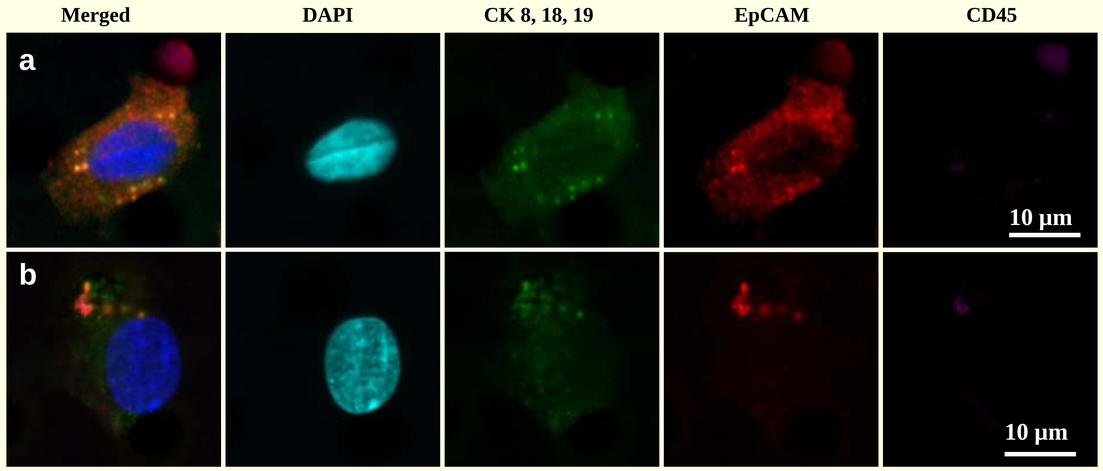


Figure 3. Clusters of EMT-Like CTC
(a) Sheet of EMT-Like CTCs with attached white blood cells
(b) Tight cluster of EMT-Like CTCs with an attached white blood cell

RESULTS

- In total 50 pancreatic cancer patient samples were run. Stage I (n=7), Stage II (n=14), Stage III (n=0), Stage IV (n=8), and Non-staged (n=21).
- Pathologically defined CTCs (CK+ and EpCAM+) were found in 24% (12/50) of samples.
- EMT-like CTCs (weak cytokeratin and low/no EpCAM) were found in 78% (39/50) of samples.
  - EMT-like cells often lacked EpCAM expression.
  - EMT-like cells commonly presented as multi-cell clusters in 44% (22/50) of samples.
- The two subtypes of CTCs overlapped in 22% (11/50) of patient samples.
- Neither cell type was found in any healthy subjects (n=30).



CONCLUSIONS

- Microfiltration captures CTCs regardless of surface marker expression.
- Pancreatic CTCs have multiple distinct phenotypes.
- Low protein expression of the EMT-like subtype implies immuno-based isolation may be limited for pancreatic cancer.
- CTC subtypes may indicate definable traits which may be exploited for personalized treatment of cancer patients.

References

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