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Mitosis in circulating tumor cells and its prognostic significance in late stage breast cancer

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ABSTRACT

It has been well documented that enumeration of Circulating Tumor Cells (CTCs) isolated from the peripheral blood of breast cancer patients can be used as a prognostic indicator of survival. CTC identification typically relies on immunohistochemical stains used in an absent/present method (i.e. CK+/EpCAM+/CD45-). However, this methodology for identification of CTCs is highly subjective, and histological cytology remains the standard identifier of cancer cells. We expand upon our work regarding the cytological criteria of CTCs, Adams et al, Cytometry 2015[1], to determine if pathological grading criteria can be applied to CTCs. We report the assessment for overall survival of 36 late stage breast cancer patients in relation to CTC number and presence of active mitosis.

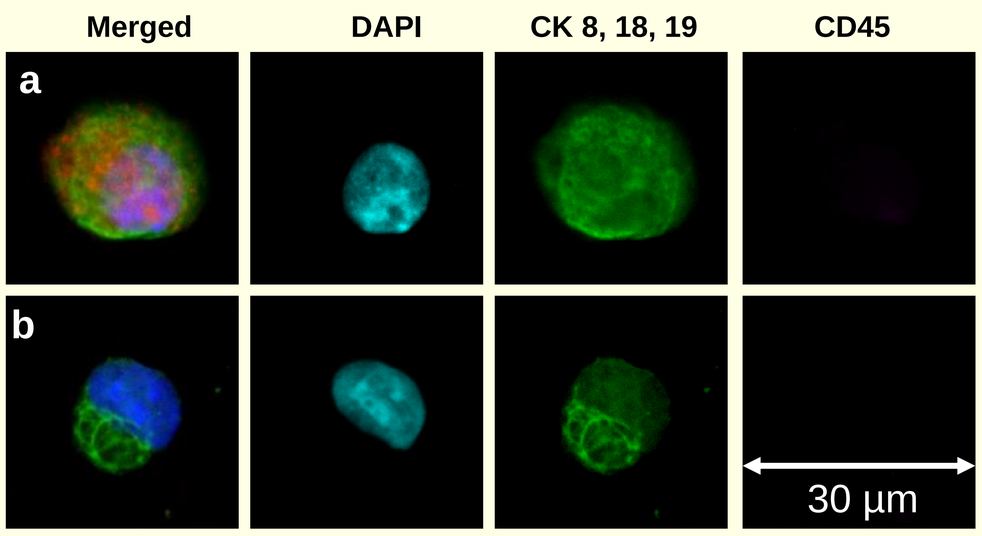

Figure 1. Examples of pathologically defined CTCs. (PDCTC) (a) PDCTC with EpCAM positivity (red) (b) PDCTC without EpCAM (absent red)

INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. CTC enumeration can be used to monitor therapy response and predict outcome.[1-4] However, CTC subtyping remains reliant on immuno-staining presence/absence, rather than the more standardized histopathological identification[1-2].

Low pressure microfiltration using CellSieve™ microfilters is a technique shown to isolate patient CTCs, while retaining the fine morphological detail required for histopathology[1-2]. High resolution morphology can identify CTC subtypes, i.e. apoptotic CTCs, highly pleomorphic CTCs, and CTCs in active mitosis. Aggressive phenotypes are associated with CTC population in mitosis. Subtyping by phenotypic determinates may aid in identifying CTCs cellular status for diagnosis, prognosis and therapy determination.[1-4]

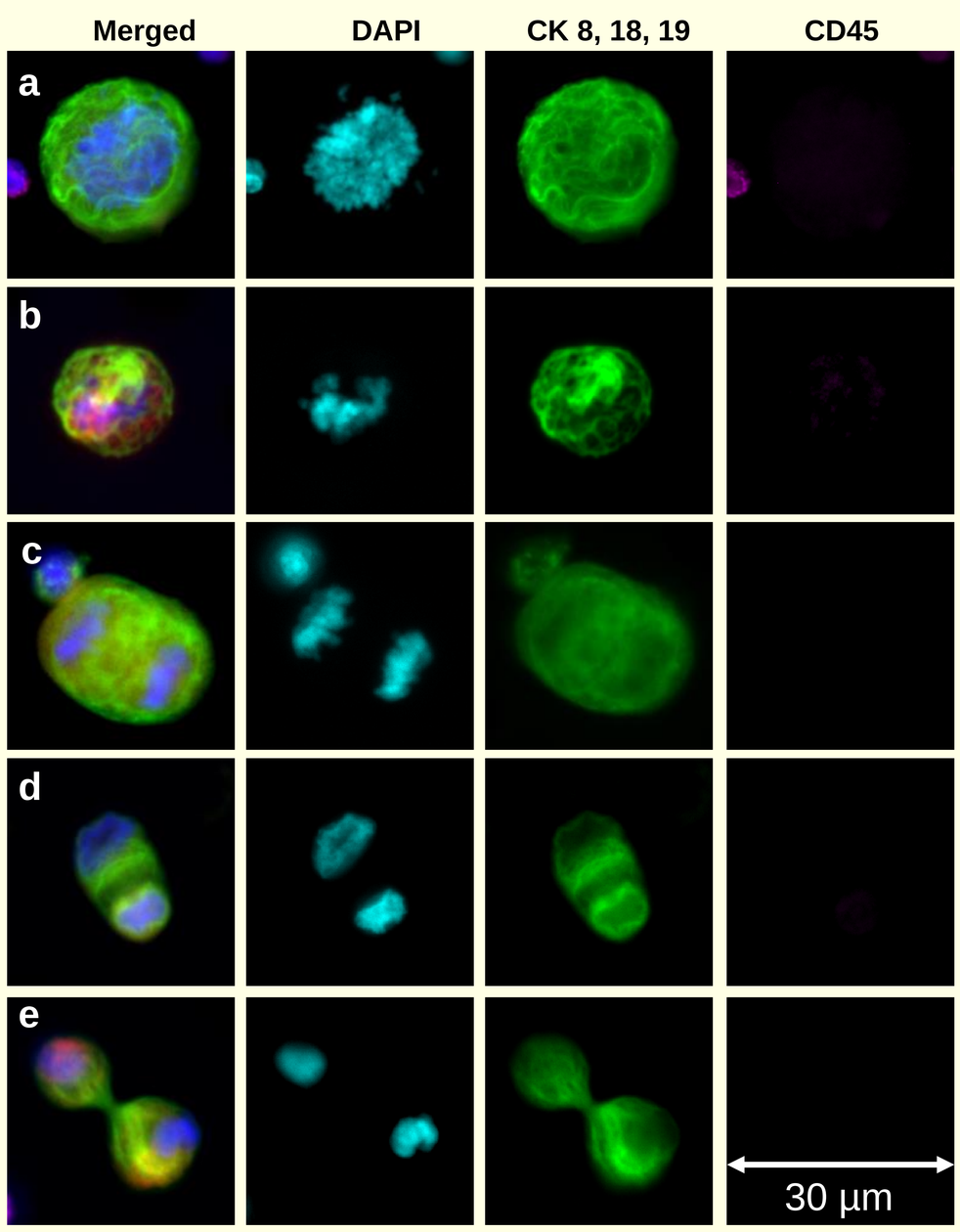

Figure 2. Examples of mitotic PDCTCs (a) Prophase, (b) metaphase, (c) anaphase, (d) telophase, (e) telophase/cytokinesis

MATERIALS & METHODS

A prospective pilot study of 36 single blinded Stage III/IV breast patient samples were provided by Fox Chase Cancer Center and University of Maryland Baltimore. 7.5mL whole blood was diluted in pre-fixation solution and filtered by CellSieve™ microfiltration. Cells were fixed, permeabilized, and stained with DAPI, an antibody cocktail against CK 8/18/19, EpCAM, and CD45. CTCs were enumerated and identified as described by Adams et al. Cytometry 2015[1]. CTCs were further subtyped by 1) number of pathologically definable CTCs (PDCTCs) and 2) presence of mitotic events, identified by standard visual cues (e.g. prophase, anaphase, etc.). Kaplan-Meier plots and Hazard ratios were determined at 24 months, with power analysis (1-β=0. 9, α=0.05) used to stratify this patient cohort.

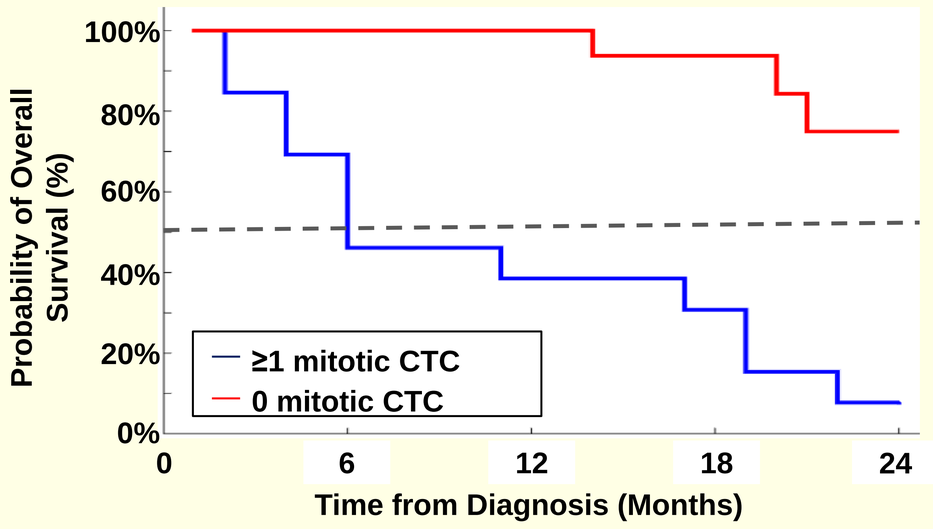

Figure 3. Kaplan-Meier Plot of patients with versus without a mitotic PDCTC event (n=36).

RESULTS

- PDCTCs were found in 83% (30 of 36) of patient samples tested.
  - 23 of 36 patients (64%) had <5 PDCTCs with a median survival of >24 months
  - 13 of 36 patients (36%) had ≥5 PDCTCs with a median survival of 10.0 months,
  - Hazard ratio was 5.2.
- Mitotic PDCTCs were found in 36% of patient samples tested
  - 23 of 36 patients (64%) had 0 mitotic PDCTCS, median survival of >24 months
  - 13 of 36 patients (36%) had ≥1 mitotic PDCTCs, median survival of 5.7 months
  - Hazard ratio was 11.1.

Variable	Hazard Ratio	95% CI	p value
1 mitotic CTC vs 0 mitotic CTC	11.1	3.1-39.7	<0.001
≥5 CTC vs <5 CTC	5.2	1.6-16.5	0.005
ER/PR positive vs negative	1.3	0.5-3.7	0.174
HER2 positive vs negative	1.8	0.6-5.7	0.289
Hormone positive vs negative	4.0	1.4-11.2	0.009

Table 1: Prediction table with the hazard ratios, confidence intervals and p-values for the patient populations

CONCLUSIONS

- Low pressure microfiltration captures CTCs while retaining fine cellular features, such as mitosis.
- Mitotic CTCs are relativity common in aggressive late stage breast cancer patients.
- Stratification of breast cancer patients based on CTCs is a prognostic indicator of survival.
- Prognostic value is dramatically increased by subtyping CTCs based on their mitotic index.
- CTC subtypes indicate definable traits that can be exploited for personalized treatment of cancer.

References

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