Characterizing and Correlating CellSieve™ and CellSearch® CTCs by Cytometric Analysis

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ABSTRACT

Recent studies reporting hundreds or thousands of circulating tumor cells (CTCs) in the blood of cancer patients have raised questions regarding the prevalence of CTCs, as enumerated by the CellSearch® test. Although CellSearch® detects clinically relevant CTCs, the ability to capture only EpCAM+ cells has led to speculation that it captures only limited subsets of CTCs. In contrast, alternative isolation approaches often capture large numbers of CTCs from patient blood samples and, not surprisingly, these alternative approaches correlate poorly with CellSearch®.

In this method comparison study we compared a microfiltration system (CellSieve™) with CellSearch®, to enumerate CTCs captured from the blood of 30 cancer patients. Like many non-EpCAM techniques, CellSieve™ isolated a greater number of Cytokeratin+ (CK+)/CD45- cells than CellSearch®, and analysis showed a low correlation between the two systems. However, by sub-grouping CK+ cells based on distinct CK staining patterns and nuclear morphologies we elucidated a subpopulation which correlated to CellSearch®. These data suggest that although various CTCs with similar phenotypic expressions are present in cancer patient blood, the clinically relevant cells isolated by CellSieve® can be isolated and identified using a non-EpCAM dependent approach.

INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. CTCs have been shown able to monitor therapy response and predict patient outcome. Size exclusion is one technique for isolating CTCs irrespective of their surface marker expression capable of isolating various subtypes of CTCs. CellSieve™ microfilters are lithographically fabricated membranes with high porosity, precise pore dimensions, and patterned distribution. We previously reported that CellSieve™ rapidly and efficiently isolates a variety of CTCs from whole peripheral blood, using fluorescent antibody stain as the detection platform. Further, it has been postulated that subtyping by phenotypic determinates may aid identifying the CTC cellular status for assay comparison, diagnosis, prognosis and therapy determination.

RESULTS

CTCs isolated by CellSieve™ express three distinct cytokeratin, histologically definable, patterns (filamentous, diffuse and punctate).

CTCs isolated by CellSieve™ can be identified as apoptotic or pleomorphic.

The total CK+/CD45- CTC group can be subtyped into 4 distinct groups: pathologically definable (PD), epithelial-to-mesenchymal (EMT), early apoptotic (EA) & late apoptotic (LA).

CK+/CD45- CTCs isolated by CellSieve™ did not correlate to CellSearch® (R²=0.44, p<0.001).

PDCTC CTCs with a filamentous CK pattern and pleomorphic nucleus (PDCTC) had a significant correlation with CellSearch® (R²=0.91, p<0.001).

CONCLUSIONS

Microfiltration captures CTCs regardless of EpCAM expression.

Microfiltration captures weakened and apoptotic CTCs.

A subtype of CK+ CTCs captured by CellSieve™ microfiltration can be statistically correlated with CTCs enumerated by CellSearch®.

The prognostic implications of CellSearch® CTCs can be applied to non-EpCAM based capture systems.

The "accepted" clinical cutoff of ≥5 CTCs/7.5 mL for CellSearch® is also a predictor of patient overall survival over a 24 month period using CellSieve™ filtration.

Table 1: Correlations of CK+/CD45- subpopulations identified by CellSieve™ filters versus CellSearch® CTCs

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<tr>
<td>R²</td>
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<td>1.40x</td>
<td>0.02x</td>
<td>1.64x</td>
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Figure 1. Subpopulations of CTCs from CellSieve™ microfilters. a) CTCs categorized as PDCTCs with filamentous cytokeratin and malignant nuclei. b) EMTCTCs with diffuse cytokeratin patterns and irregular nuclear patterns. c) EACTC with punctate cytokeratin and malignant nuclei d) LACTC with punctate cytokeratin and blebbing nuclei.

Figure 2. Representative examples of method comparisons and clinical outcomes for three CTC subtypes identified by CellSieve™ versus CTCs enumerated by CellSearch®.

References