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. Using a microfiltration approach, we show that two distinct CTC subtypes can be identified in the peripheral blood in 80% of pancreatic patients.

RESULTS

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. Size exclusion is a technique for isolating CTCs from patient samples, irrespective of their surface marker expression. 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.

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. Size exclusion is a technique for isolating CTCs from patient samples, irrespective of their surface marker expression. 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.

Material & 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.

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