Redefining CTCs: Detection of cytokeratin-negative circulating tumor cells (CTCs)

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Abstract

Background: There is growing evidence that CTCs can display an epithelial-mesenchymal transition phenotype. Current enrichment techniques rely upon epithelial markers for capture and detection (anti-EpCAM antibodies and cytokeratin [CK]) and may miss important populations of circulating tumor cells. We sought to develop a new method for identification of CTCs.

Methods: After IRB approval, blood from patients with ovarian or colorectal carcinoma was collected. Cell Enrichment and Extraction (CEE) technology, a microfluidic-based device using an antibody cocktail targeting epithelial and mesenchymal cell surface markers, was utilized for the capture and analysis of rare cells in blood. We enumerated cells that were CK+ and/or contained complex aneuploidy by fluorescence in situ hybridization (FISH). Fresh and frozen tumor samples were analyzed on a subset of patients to evaluate concordance of molecular alterations with CTCs. An orthotopic ovarian cancer model was used to evaluate effects of chemotherapy on CTCs.

Results: Enumeration of CK+ cells identified an average of 1.5 cells per 10 mL blood in ovarian and colorectal cancer patients, independent of stage or tumor grade. Enumeration did not correlate with serum tumor marker levels. The majority of CK+ cells had complex (>2 abnormalities) aneuploidy. Ovarian cancer patients had equal numbers of CK- and CK+ complex aneuploid cells (p=1.0). A three-fold increase in CK- over CK+ complex aneuploid cells was noted in patients with colorectal cancer (p=0.19). Similar patterns of complex aneuploidy were identified in patients’ primary tumors as were seen in their CK- cells from blood, indicating that the cells identified in circulation were indeed CTCs. No CK+ or complex aneuploid cells were found in healthy volunteers. In the orthotopic mouse model, compared to tumor-bearing controls, chemotherapy treatment initially lead to a 17-fold increase in apoptotic appearing CTCs (p=0.02) followed by a 15-fold decrease in CTCs (p=0.03). Discussion: We have developed and characterized a novel and robust method for detection of CTCs. The power of this approach lies in its ability to detect CTCs selectively and enrich for CTCs. The inclusion of a glass coverslip as part of the CEE device allows direct addition to immunochemical and genetic analysis, using standard microscopy. The polymer chip consists of ~9000 posts of variable size/diameter and placement.

Conclusions: We have developed a robust method for detecting CTCs that can capture and identify both epithelial and mesenchymal phenotypes. Our findings suggest current enrichment techniques may be missing important populations of cells.