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

Chad V. Pecot[1], Farideh Bischoff[2], Yvonne Lin[3], Padmavathi Jaladurgam[1], William Merritt[4], Tony Pircher[2], Steve Mikolajczyk[2], Julie Mayer[2], Karina Wong[2], Tam Pham[2], Justin Bottsford-Miller[1], Rebecca Stone[1], Joseph Celestino[1], Alpa Nick[1], Cathy Eng[1], Anil Sood[1]

UT M.D. Anderson Cancer Ctr., Houston[1], TX; Biocept Incorporated[2], San Diego, CA; University of Southern California[3], Los Angeles, CA; South Carolina Oncology[4], Columbia, SC

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

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 and 2 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 with either an epithelial or mesenchymal phenotype as expected in EMT. Our findings suggest current enrichment techniques may be missing important populations of cells.

INTRODUCTION

- Current enrichment techniques rely upon epithelial cell surface markers for capture, and may miss important populations of CTCs
- We sought to develop a new method of capturing epithelial and mesenchymal CTCs

METHODS

- Blood from patients with ovarian or colorectal cancer was collected and CTCs were isolated using Cell Enrichment and Extraction technology
- CTCs were enumerated based on either CK staining and/or complex aneuploidy by fluorescence *in situ* hybridization
- Fresh/frozen tumor samples were analyzed on a subset of patients
- An orthotopic ovarian cancer model was used to evaluate effects of chemotherapy on CTCs

RESULTS

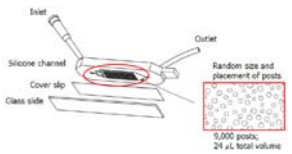

Figure 1. The CEE™ platform utilizes a microfluidic chip to selectively capture and enrich for CTCs. The inclusion of a glass coverslip as part of the CEE™ device allows direct and immediate visual assessment of captured cells, in addition to immunochemical and genetic analysis, using standard microscopy. The polymer chip consists of ~9000 posts of variable size/diameter and placement.

Cytokeratin-Positive CTCs

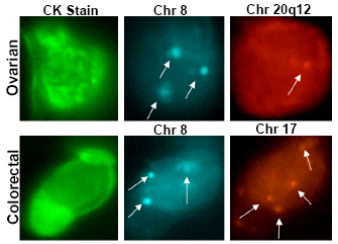

Figure 2. Staining and detection of CK-negative circulating ovarian (top) and colorectal (bottom) carcinoma cells. FISH staining shows an ovarian cancer cell with trisomy in chromosome 8 (blue) and monosomy in region 20q12 (red), whereas the colorectal cancer cell has trisomy in chromosome 8 and tetrasomy in chromosome 17 (orange, arrows).

Cytokeratin-Negative CTCs

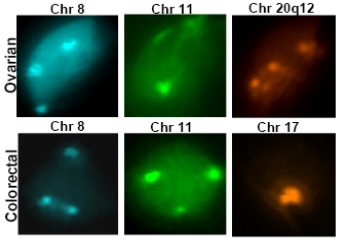

Figure 3: Detection of cytokeratin-negative circulating ovarian (top) and colorectal (bottom) carcinoma cells. FISH staining an ovarian cancer cell with trisomy in chromosome 8 (blue), monosomy in chromosome 11 (green) and tetrasomy in region 20q12 (orange), whereas the colorectal cancer cell has trisomy in chromosomes 8 (blue) and 11 (green) as well as monosomy in region 20q12.

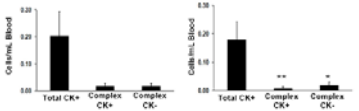

Figure 4: Average number of total cytokeratin, complex aneuploid cytokeratin-positive and cytokeratin-negative circulating tumor cells per milliliter of blood for ovarian (left) and colorectal (right) cancer patients. *p< 0.05, **p=0.01

Cytokeratin-Negative Cells in the Primary Tumor

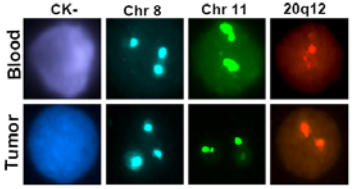

Figure 5: Cytokeratin-negative ovarian cancer cells identified in circulation at the time of surgical resection have similar aneuploidy as regions in the tumor.

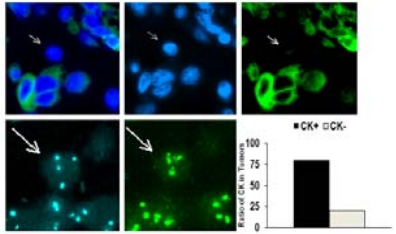

Figure 6: Cytokeratin staining of ovarian carcinoma tumors reveals cytokeratin-negative cells with aneuploidy (arrows) similar to those detected in circulation. Approximately 20% of the tumors had such cytokeratin-negative complex-aneuploid positive cells.

Therapeutic effects on CTCs

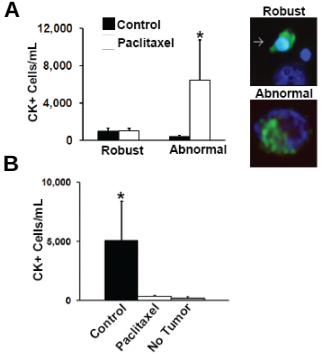

Figure 7: (A) In an orthotopic ovarian carcinoma model, blood samples were obtained 3 days after administration of paclitaxel. Robust-appearing cells were about the same in treated *versus* untreated mice. A significant increase in apoptotic (abnormal)-appearing cells in the treatment group was observed (right). (B) The number of robust-appearing cytokeratin-positive cells at the time the mice were killed was significantly higher in the control group than in the treatment group and in mice without tumor.

CONCLUSIONS

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