Fluorescence labeling of cytokeratin-negative circulating tumor cells in peripheral blood: A new paradigm for the study of cancer progression

Stephen D. Mikolajczyk, Lisa S. Millar, Maryam Zomorrodi, Farideh Z. Bischoff, Jayne Scoogg, Tam Pham, Karina Wong, Tony J. Pircher

Biocept Inc., San Diego, CA 92121

ABSTRACT

Introduction: The enumeration of circulating tumor cells (CTCs) in blood generally requires either cell-DR impedance for capture, anti-epithelial cell adhesion molecule (EpCAM) antibodies, or a combination of both. Using cell-DR technology, we previously captured cancer cells from blood samples, and using the monoclonal antibody (mAb) to carcinoembryonic antigen (CEA) and cytokeratin (CK) using a microfluidic channel. A new paradigm for the study of cancer progression is under investigation.

Objective: The objective of this study was to evaluate CEE-Enhanced™ technology to detect cells retained on the channel with the capture antibodies bound to the cell surface antigens. All cells scored as positive for CK or CE were, by definition, CD45-negative and DAPI-positive.

Methods: Buffy coat cells were prepared from blood using a density gradient. Capture antibodies were then incubated with the Buffy coat cells and then reacted with biotinylated streptavidin conjugated to a fluorescent dye. The channel was then re-stained with CE. The same cell after subsequent enhancement is shown.

Results: The CEE-Enhanced™ technology greatly expands single and multi-antibody approaches to the study of rare circulating cells.

CEEMicro-channel

A) Top view of the channel showing the inlet where sample is loaded and the outlet that is attached to a syringe pump to draw sample through the channel. B) Bottom view shows the area where 9,000 posts are located in the silicon block and the channel sealed with the bottom cover slip. The total volume of the micro-channel is 24 μL. The microscope slide is added for stability during handling but is removed to visualize cells. The micro-channel is inverted on a microscope and the captured cells viewed through the coverslip.

Visible light microscopic view of channel through the bottom cover slip showing the random area of posts and SKBR3 cells which have been captured (sense).

RESULTS

Table 1: CTC capture using EpCAM-only and an antibody mixture (CE-Enhanced™) in peripheral blood samples. All cells were CK+ or CK- and DAPI+.

Sample 1  2  3  4  5  6  7  8  9  10  11  12

Buffy coat 29  17  4  2  1  1  1  1  1  1  1  1

CK+ 12  7  1  1  1  1  1  1  1  1  1  1

CK- 7  10  0  0  0  0  0  0  0  0  0  0

DAPI+ 2  2  2  2  2  2  2  2  2  2  2  2

DAPI- 27  15  3  1  1  1  1  1  1  1  1  1

Additional CTCs were detected using CEE-Enhanced™ that are not detected with anti-CK alone alone. Greater numbers of CEE-Enhanced™ CTCs were detected when using an antibody mixture compared to EpCAM alone.

Table 2: The use of CEE-Enhanced™ (CE) to improve detection of CK-positive cells on the micro-channel.

Sample 1  2  3  4  5  6  7  8  9  10  11  12

Buffy coat 29  17  4  2  1  1  1  1  1  1  1  1

CK- 7  10  0  0  0  0  0  0  0  0  0  0

DAPI+ 2  2  2  2  2  2  2  2  2  2  2  2

DAPI- 27  15  3  1  1  1  1  1  1  1  1  1

Results: The CEE-Enhanced™ technology greatly expands single and multi-antibody approaches to the study of rare circulating cells.

CONCLUSIONS

- Antibody mixtures improve the recovery of cancer cells, including CTCs not captured with anti-EpCam alone
- CEE-Enhanced™ can be used to stain CTCs that do not stain with anti-cytokeratin
- CEE™ technology allows multiple screening and staining strategies for the analysis of CTCs

Staining clinical lung cancer CTCs with anti-CK and CEE-Enhanced™

A. CTC on the micro-channel stained with anti-CK (green). B. The same CTC co-stained with CEE-Enhanced™ (AlexaFluor 647, orange). C. Cluster of CTCs stained with anti-CK.

Staining breast cancer CTCs sequentially stained with anti-CK and CEE-Enhanced™ (CE) An antibody mixture was used to capture CTCs. CK-positive cells were detected in a sequential series of stage IV breast cancer samples. The locations of these cells were recorded and then the channel was re-stained with CE. The stacked upper lane represents the new CTCs detected with CE. All cells detected as labeled-positive were CK- and DAPI-positive.