Detection and Characterization of CTCs Isolated by ScreenCell® Size Exclusion Technology in Metastatic Breast Cancer

Zhaomei Mu[1], Naoual Benali-Furet [2], Georges Uzan[2], Zhong Ye[1], Carmela Paolillo[1], Laura Austin1, Chun Wang[1], Rebecca Jaslow[1], Hushan Yang[1], Paolo Fortina[1], Massimo Cristofanilli[1]

[1]Thomas Jefferson University and Sidney Kimmel Cancer Center, Philadelphia, PA, USA, [2]ScreenCell, Sarcelles, France

Background

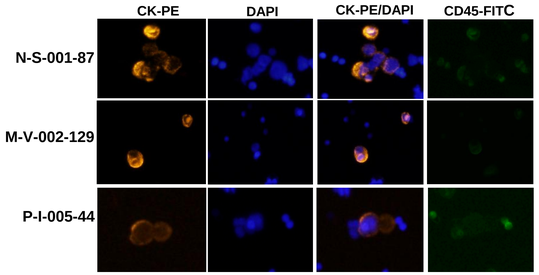
Circulating Tumor cells (CTCs) detection has prognostic and predictive implications in patients with metastatic breast cancer (MBC). Genomic and phenotypic analysis of CTCs hold enormous promise as blood-based molecular characterization and monitoring disease progression and treatment benefit with a strong potential to be translated into more individualized targeted treatments. FDA-approved CellSearch™ detection allows only enumeration of CTCs expressing EpCAM. CTCs represent very heterogeneous populations of tumorigenic cancer cells and some subpopulations have undergone epithelial-Mesenchymal transition (EMT), which is associated metastasis process and an unfavorable outcome. EpCAM-based enrichment technique has failed to detect EMT subpopulations due to the decreased expression or loss of epithelial markers. Non-EpCAM-based approaches are needed for identifying EMT CTCs. The ScreenCell® devices are single-use and low-cost size exclusion technology for enrichment-free isolation of CTCs by a two-steps combining size-based separation and staining using different markers. The DEPArray™ system is the ideal downstream isolation system to collect single or pooled CTCs for molecular and genetic analysis. In this study, we evaluated CTCs, CTC-clusters and EMT CTCs, and tested the feasibility of achieving CTCs enrichment using ScreenCell® technology followed by single cell isolation with the DEPArray™ in MBC patients.

Material & Methods

1. CTCs and CTC-clusters detection/enumeration was evaluated in 30 patients with MBC. 3 mL of whole blood in an EDTA or Transfix tubes was collected and processed on the ScreenCell® Cyto device following the instructions of the supplier. CTCs and CTC-clusters were stained with cytokeratin (CK-8, 18, and 19 from Janssen), leukocyte antigen (CD45), and a nuclear dye (DAPI) and counted under fluorescence microscope. EMT CTCs were stained with Vimentin-FITC (Santa Cruz) and N-Cadherin-FITC (BioLegend).
2. After enrichment by ScreenCell®-Cyto device, CTCs were stained with CK, CD45, and DAPI and sorted with DEPArray™ Platform (Silicon Biosystems, Inc). Single CTCs were collected and the DNA of each single CTCs was amplified with Ampli1™ Whole Genome Amplification (WGA) kit, and the genome integrity index (GII) was assessed by Ampli1™ QC kit (Silicon Biosystems, Inc). Detection of activating Estrogen Receptor 1 (ESR1) and TP53 mutations was performed on ABI PRISM® 3700 genetic analyzer by target Sanger sequencing.

Results

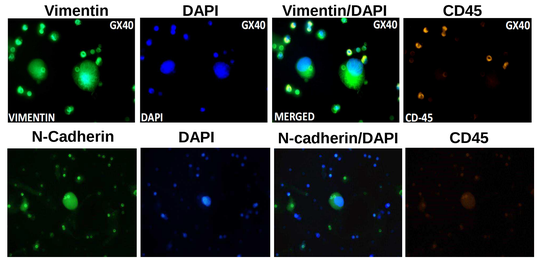
Figure 1. CTCs and CTC-clusters were detected in the blood from metastatic breast cancer patients



CTCs and CTC clusters were identified as positive staining for Cytokeratin (CK) and DAPI and negative staining for CD45 (CK+/DAPI+CD45-), the images from 3 MBC patient samples.

Results

Figure 2. Vimentin and N-Cadherin expression in CTCs of metastatic breast cancer patient samples



EMT CTCs were identified as positive staining for Vimentin-FITC or N-Cadherin-FITC and DAPI and negative staining for CD45 (Vimentin+/N-Cadherin+/DAPI+CD45-).

Figure 3. Sample processing flow on ScreenCell® device and DEPArray platform

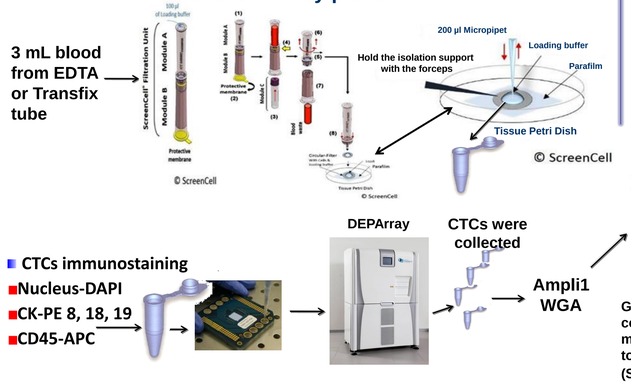
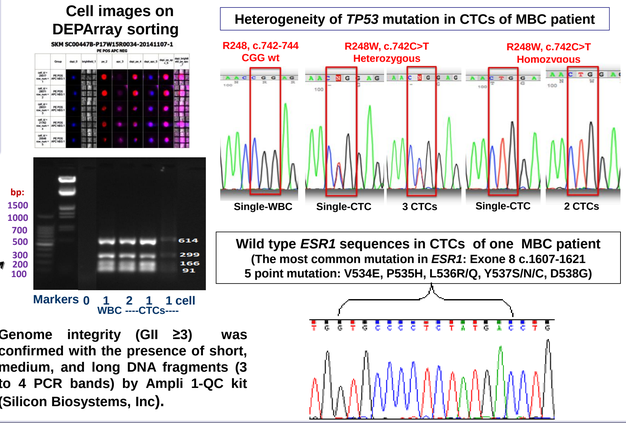


Figure 4. WGA and genome analysis-Sanger sequencing



Genome integrity (GII ≥3) was confirmed with the presence of short, medium, and long DNA fragments (3 to 4 PCR bands) by Ampli 1-QC kit (Silicon Biosystems, Inc).

Results

Table 1. Summary of CTCs and CTC-clusters enumeration in MBC patients

Total patients (N)	CTCs (≥ 1 CTCs)/3 mL N (%)	CTC-clusters (≥ 2 CTCs)/3 mL N (%)
30	20 (66.7)	8 (26.7)

Table 2. Summary of EMT CTCs in MBC patients

Vimentin+ patient /total patients (≥ 1 CTCs)/3 mL N (%)	N-Cadherin+ patient /total patients (≥ 1 CTCs)/3 mL N (%)
13/18 (72.2%)	8/10 (80%)

genome integrity index (GII) was assessed

Conclusions

1.CTCs and CTC-clusters were captured by ScreenCell® size exclusion technology without EpCAM selection in MBC.
2.EMT CTCs were identified by Vimentin and N-Cadherin staining in MBC.
3. It is feasible that CTCs can be enriched by ScreenCell® size exclusion technology followed by single cell isolation using DEPArray™ platform to achieve single cell genomic analysis.

References

1. Desitter I etal, Anticancer Res. 2011 Feb;31(2):427-41
2. Klein CA, et al. *Proc Natl Acad Sci USA* 1999 96: 4494 4499
3. Mu Z et al. Breast Cancer Res Treat. 2015 Dec;154(3):563-71

QR Code