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The Combination of the Parsortix™ System and CellCelector™ Micromanipulator Enables the Characterization of EpCAM*neg* Circulating Tumor Cells in Breast Cancer

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Aim

Nowadays CTCs are mainly enriched by EpCAM-based technologies which cannot detect the most malignant cells. In this project we established a workflow to enrich, detect and isolate EpCAM*negative* CTCs by combining potentials of both the Parsortix™ system (label-independent technology) and the CellCelector™ micromanipulator for further molecular characterizations.

Methods

A cohort of 22 metastatic breast cancer blood samples was processed through the CellSearch® system to enrich for EpCAM*positive* cells. The discarded fraction was further processed through the Parsortix™ system to enrich for EpCAM*negative* cells, based on size. Captured cells were stained *in situ* for nuclei, EpCAM, cytokeratins and CD45 to identify epithelial cells and were then harvested in a tube.

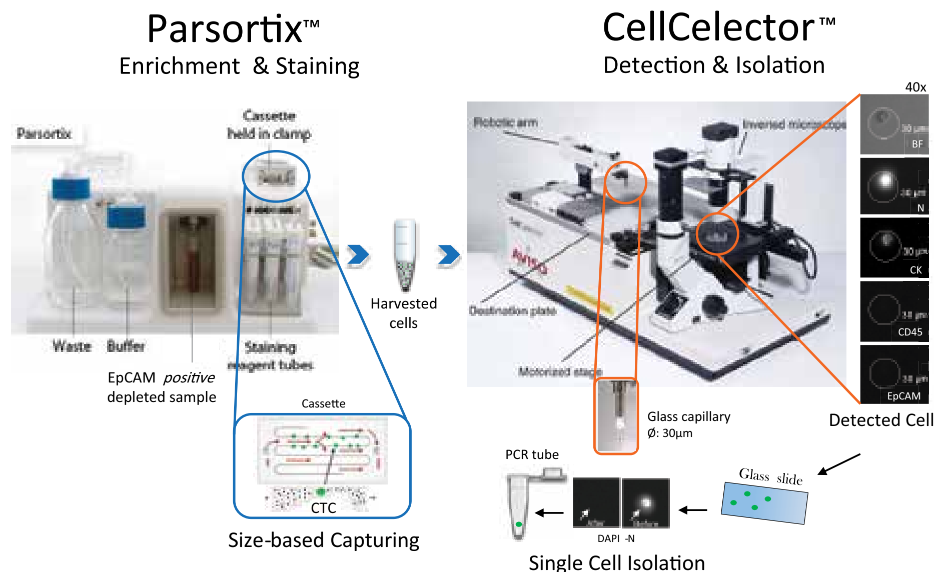
Potential CTCs were detected by immune fluorescence microscopy (nuclei+, EpCAM-, CK+, CD45-) and isolated in PCR tubes with the CellCelector™ micromanipulator. Single cells were processed for Whole Genome Amplification (*Ampli*1™) and samples with high genomic integrity were selected for the array-based comparative genomic hybridization (aCGH) in order to confirm their malignant origin.

Results

Both EpCAM*positive* and EpCAM*negative* cells were detected in the 71% of processed blood samples, and no correlation in positivity rates was observed. We were able to successfully isolate 156 EpCAM*negative* cells as well as EpCAM*positive* ones.

The WGA showed a high integrity genome in 37% of processed EpCAM*positive* cells vs 7% in EpCAM*negative* ones. This difference needs to be further investigated.

The aCGH profile of the first processed EpCAM*negative* cell was compared with 269 databased profiles of breast cancer cells and typical aberrations were observed, confirming our methods. Some different aberrations were observed as well, and they require further investigations.



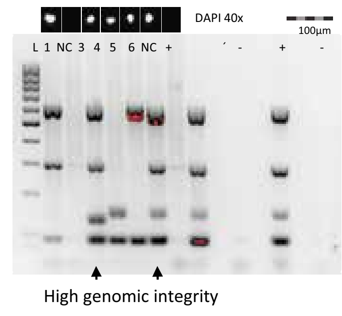
	Detected cells			Isolated cells	
Blood samples	Cells	EpCAM*neg* Cells		EpCAM*pos* Cells	EpCAM*neg* Cells
22	541	257		94	156

- 71% of samples positive for both EpCAM*pos* and EpCAM*neg* cells
- Successful isolation of both EpCAM*pos* and EpCAM*neg* cells

Processed cells

EpCAM*pos* Cells	EpCAM*neg* Cells
65	111

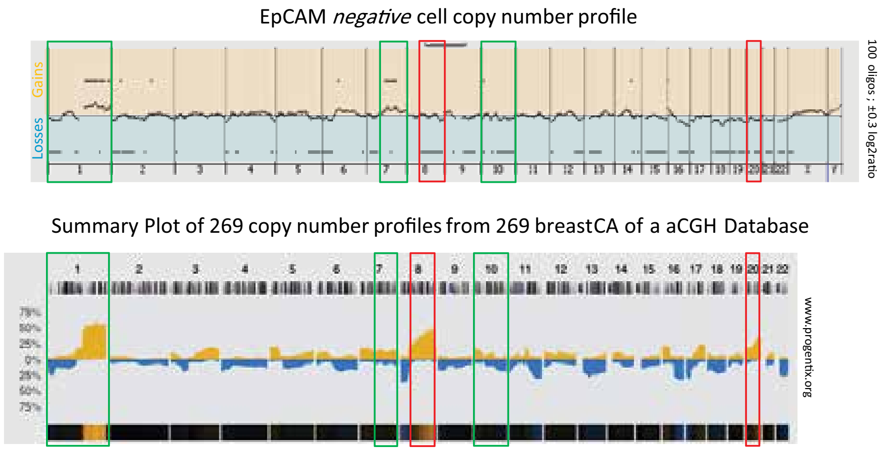
Whole Genome Amplification



EpCAM*pos* Cells high DNA integrity	EpCAM*pos* Cells low DNA integrity
24 (37%)	13 (20%)
EpCAM*neg* Cells high DNA integrity	EpCAM *neg* Cells low DNA integrity
8 (7%)	49 (44%)

- EpCAM*pos* cells show higher genomic integrity than EpCAM*neg* cells

Array-based Comparative Genomic Hybridization



EpCAM *negative* cell copy number profile

Summary Plot of 269 copy number profiles from 269 breastCA of a aCGH Database

- Hint of malignancy of the EpCAM*neg* cell

Conclusion

Our workflow allows to successfully isolate EpCAM*negative* CTCs from EpCAM*positive* depleted samples, and to further process them for molecular characterizations.

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