Highly Multiplexed Molecular Analysis of Circulating Tumor Cells Enriched Using the Epitope Independent Parsortix™ System

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Introduction

Circulating tumor cells (CTCs) represent a potentially rich source of information for cancer diagnostics and therapeutic decision-making. Molecular characterization of CTCs may help in the identification of tumor histotype, transition to invasive phenotypes, and/or mutation status. To fully exploit this non-invasive diagnostic resource requires approaches to highly enrich for tumor cells relative to other nucleated blood cells and to simultaneously detect numerous low abundance analytes.

Methods

We have coupled two technologies to enable multiplex gene expression profiling of CTCs: 1) the Parsortix™ system for epitope-independent CTC enrichment, and 2) the Axela Hybrid Capture, Enrichment, Amplification and Detection (HyCEAD) chemistry and Flow-Through Chip (FTC) hybridization system for multiplexed molecular analysis.

CTC Enrichment with the Parsortix™ system

CTCs are separated from other cells in the blood on the basis of cell size and deformability. A blood sample is continuously perfused through the Parsortix™ cartridge where CTCs are trapped while red blood cells and white blood cells pass through (Fig. 1).

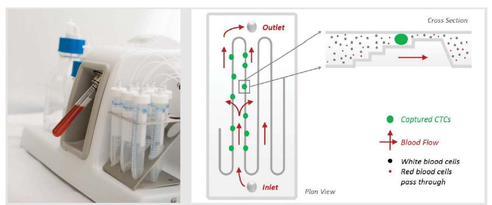

Fig. 1 – Parsortix™ instrument and the mechanism of separation.

Multiplex mRNA amplification and detection with HyCEAD

Cells harvested from the Parsortix™ cartridge in saline (PBS) are lysed to release and stabilize mRNA. Gene-specific primers are hybridized to selected mRNA targets in the lysate, and the mRNA/primer hybrids are captured by their poly-A tails (Fig. 2). Solid phase reverse transcription displaces single stranded cDNA into the supernatant. The cDNA is amplified by PCR with a mixture of gene-specific and universal primers. PCR products are hybridized on a FTC to quantify expression levels with chemiluminescence.

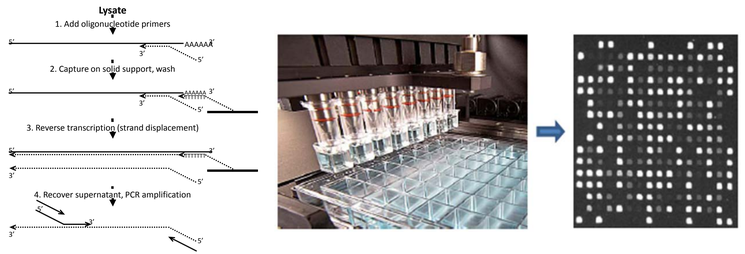

Fig. 2 – Multiplex amplification of specific mRNAs and FTC quantification.

Procedures

To assess the efficiency of recovery of cancer cells from blood with the Parsortix™ system varying numbers of Sk-Br-3 breast cancer cell line cells were spiked into 5 mL of blood from Healthy Normal Volunteers (HNVs) and processed on the Parsortix™ system. Recovered Sk-Br-3 cells were enumerated with immunofluorence with an anti-ERBB2 antibody, and the percent recovery was calculated.

Varying amounts of RNA from cancer cell lines were processed with HyCEAD to assess the sensitivity of detection and the quantitative nature of the response.

To assess the feasibility of molecular characterization of tumor cells recovered from blood, samples from HNV were spiked with 0, 10 or 100 CaOV3 cells per 10 mL of blood and processed on the Parsortix™ system to obtain enriched CTC preparations in 210 µL of PBS. Sixty mRNA targets were quantified in lysates with a 64-plex HyCEAD assay.

Results

Efficient epitope-independent recovery of CTCs from blood

The mean recovery (three different instruments) of 10 to 100 Sk-Br-3 cells was 90% (Fig. 3).

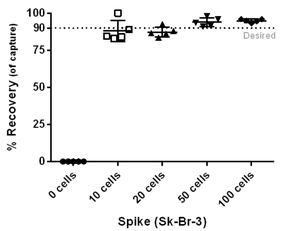

Fig. 3 – Recovery of varying numbers of spiked Sk-Br-3 from 5 mL of blood.

Multiplex expression profiling in ovarian cancer cells recovered from blood

Multiplexed gene expression analysis of cancer cell lines showed that the HyCEAD/FTC system was sensitive at the single cell level (Fig. 4) and that signal intensities scaled with the amount of input RNA (Fig. 5).

Responses of many probes were specific to cancer cells at the 1 cell/mL of blood level (Fig. 6). Approximately one half of the 64 probes analyzed had low expression levels in WBCs compared to ovarian cancer cells. Samples spiked with 10 or 100 CaOV3 cells were separated from unspiked samples by principle components analysis with a subset of the 64 probes (Fig. 7).

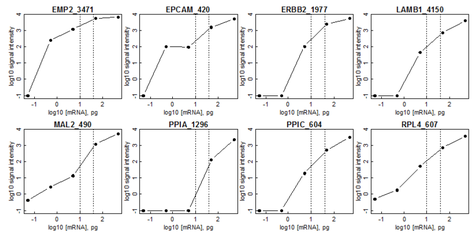

Fig. 4 – Responses of eight mRNA probes to varying amounts of CaOV3 RNA. The approximate range of RNA in a single cell is indicated by dotted lines.

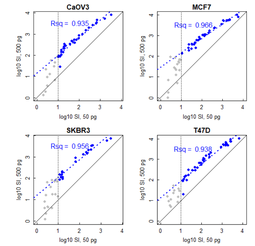

Fig. 5 – Correlation of expression levels for 64 mRNA probes with 10-fold increase of input mRNA. Probes with significant expression at 50 pg are shown in blue.

Gene #	mean.unspiked	mean.10.cells	pvalue
1	269.7	4000	0.0002
2	64.3	1161	0.0005
3	64.8	2657	0.0010
4	201.4	2316	0.0025
5	18.7	497	0.0075
6	5.5	224	0.0094
7	7.2	395	0.0094
8	7.9	59	0.0143
9	15.1	244	0.0192
10	59.1	553	0.0203
11	5.9	238	0.0226
12	16.7	55	0.0244
13	49.2	312	0.0262
14	36.9	1247	0.0305
15	1.2	91	0.0320
16	1.4	18	0.0392
17	1.3	11	0.0623
18	6.1	42	0.0735
19	2.5	32	0.0760

Fig. 6 – Mean expression of selected genes in Parsortix™ harvests from HNV blood without and with spikes of 10 CaOV3 cells/10 mL and the p-value (N= 8).

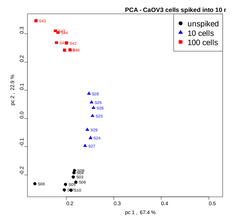

Fig. 7 – Principle components analysis of 10 mL blood samples from HNVs spiked with either 10 or 100 CaOV3 cells.

Conclusions

The combination of CTC enrichment with the Parsortix™ system, which allows recovery of small numbers of spiked CTCs with low WBC contamination, and multiplexed gene expression analysis with the Axela HyCEAD/FTC system, which enables the simultaneous evaluation of a large number of genes, shows great promise both as a clinical research tool to discover informative biomarkers and as an efficient system for development of highly multiplexed diagnostic tests in ovarian cancer and other solid tumors.