Isolation Of Pure Tumor Cells From Peripheral Blood Using MagSweeper Enrichment

The isolation of pure and viable circulating tumor cells (CTC) from blood samples represents a great potential for the development of personalized approaches in the oncologic field. To-date several techniques have been proposed for the isolation of CTCs, each obtaining good results in terms of sample enrichment, but none able to get pure cell populations. Starting from a small pool of enriched cells, the DEPArray™ system is able to isolate single cells with 100% purity. Here we demonstrate the compatibility of the DEPArray™ system with the MagSweeper instrument, a new immunomagnetic cell separator reportedly able to enrich both fixed and live target cells (Ref. Talasaz A. H. et al., PNAS 2009). The purity of cells isolated by DEPArray™ technology was demonstrated through genomic sequence analysis of the recovered cells.

Materials and Methods

A healthy donor sample spiked with a colorectal cancer cells carrying a K-Ras mutation, in blind, was enriched with the MagSweeper system selecting for EpCAM positive cells (courtesy Fox Chase Cancer Center, Philadelphia). Labeling was performed with EpCAM-PE, CD45-FITC, and Hoechst to allow the discrimination between target cells and control cells. The enriched sample was prepared for analysis and cell recovery on the DEPArray™ system according to Silicon Biosystems' standard procedure. In the experiment multiple recoveries of PE-positive target cells as putative tumor cells were performed. Multiple individual tumor cells were recovered and their DNA was amplified using the *Ampli*1™ WGA kit to obtain the entire genome of single cells. A PCR amplification for exon 2, codons 12-13 of the K-Ras gene was performed using gene specific primers on a small aliquot of the *Ampli*™ WGA product. The amplicon was sequenced in order to analyze the mutational configuration present. The experimental scheme is shown in Figure 1.

Results

Seven (7) single EpCAM+/CD45-/Hoechst+ cells were isolated as putative colorectal cancer tumor cells (see an example in Figure 2). The bright field images obtained clearly show the presence of several dark beads bound to target cells. Beads form a dark cluster around the cells but they did not prevent the detection of target cells from contaminant cells. Moreover, the beads did not impair the manipulation and recovery of cells with DEPArray™ technology. Sequencing of the amplified cells showed a heterozygous mutation of G13D in codon 13 of the K-Ras gene, Figure 3. The mutation was easily detected in all of seven single tumor cells isolated thanks to the absence of wild type DNA from contaminating leukocytes and the well balanced amplification from *Ampli*1™ WGA. The mutation was identified in heterozygosity in 6/7 (85%) of the samples, the single homozygous sample might be due to either single-cell heterogeneity or an allelic drop-out during the WGA amplification.

Conclusions

The flexibility of the DEPArray™ system allows compatibility with selective positive enrichment on the MagSweeper system making real the possibility to obtain single pure cells. The excess of beads bound to target cells didn't affect the result in terms of cell identification, isolation and manipulation. After the recovery, cell characterization at the genetic level demonstrates the 100% purity of recovered target cells, confirmed also by the detection of the heterozygous mutation.

Keywords: DEPArray™ ; MagSweeper, Colorectal Cancer; single pure cell.

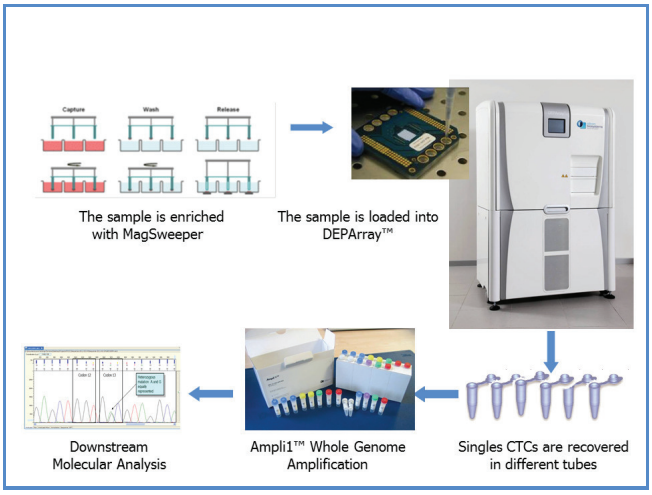

Figure 1: Experimental work-flow.

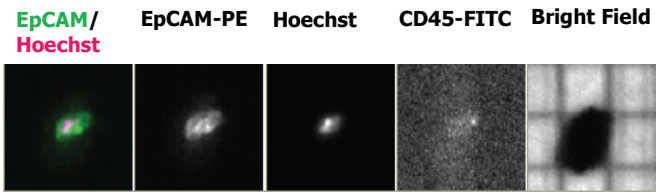

Figure 2: Image gallery from the DEPArray™ CellBrowser software showing one cell recovered after MagSweeper enrichment and isolation on a DEPArray™ system. The CTC are characterized by the presence of EpCAM and the absence of CD-45.

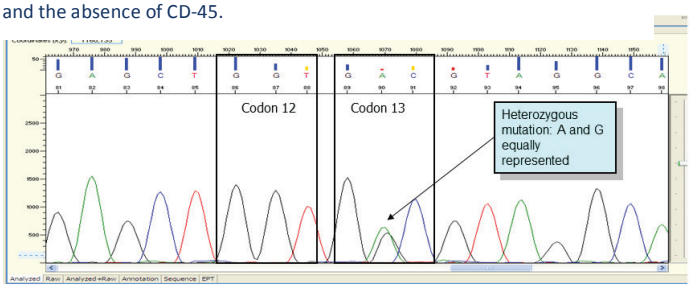

Figure 3: Electropherogram obtained after sequencing of K-Ras gene of recovered cell number 4. The codon 13 showed the mutation G13D.

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