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Application Note

DEPArray™ Rare Cell Isolation Technology

Isolation and Molecular Characterization of Pure CTCs from Blood Samples Enriched with CellSearch® System

Introduction

The isolation and molecular characterization of circulating tumor cells (CTCs) has a great potential for serially monitoring the molecular profile of a tumor. Several techniques have been developed for CTC enrichment and enumeration from peripheral blood. Veridex, with its CellSearch® System, demonstrated the relevance of CTCs associated with various different solid tumors by showing they are prognostic regarding patient overall survival. The CellSearch® System obtained FDA clearance for the enumeration of CTCs in metastatic Breast, Colorectal and Prostate cancers. The CellSearch® System has been widely adopted as it automates and standardizes sample collection, enrichment and staining of CTCs from blood. However, as with all other enrichment systems, the low purity provided by CellSearch® System makes it impractical to carry out the molecular analysis of the CTCs detected, and to characterize their heterogeneity which will be required to advance personalized treatment of patients. We show here that the DEPArray™ system overcomes these limitations, achieving 100% purity in the isolation of a mixed population of tumor cells from blood samples after enrichment using the CellSearch® Autoprep. Thus, the DEPArray™ system, when used in combination with single-cell whole genome amplification by the *Ampli*1™ WGA kit, is able to reliably detect mutations in TCs and characterize their molecular heterogeneity (**Fig.1**).

Materials and Methods

Healthy-donor peripheral blood samples collected in CellSave tubes were spiked with viable KRAS-mutated tumor cell lines (SW480 colorectal cancer cell line; A549 lung cancer cell line) and enriched for TCs on Veridex's AutoPrep with the CellSearch® Epithelial Cell Kit. Samples were extracted from the CellSearch® cartridge and prepared for sorting and analysis on a DEPArray™ system according to Silicon Biosystems' standard procedure. Multiple individual pure collections of single TCs and control WBCs were recovered along with small cell pools. For each of the recovery tubes *Ampli*1™ WGA Kit (Silicon Biosystems) was used for cell lysis and whole genome amplification. The resulting *Ampli*1™ DNA library was fingerprinted by Short Tandem Repeats (STR) analysis. Further, KRAS gene-specific products were sequenced.

Results

Cells were analyzed on the DEPArray™ system to isolate and recover pure tumor cells or leukocytes (WBCs) for molecular analysis. The cell type(s) of tumor cells spiked in each sample was blinded to the laboratory staff collecting and analyzing the cells. An image-based selection of all the events detected by the DEPArray™ system allows one to clearly identify and selectively chose for sorting only best and pure TCs (CK+/CD45-/DAPI+) (**Fig.2**) and WBCs (CK-/CD45+/DAPI+). Across four spiking experiments (SW480 n=2, A549 n=1, mix SW480/A549 n=1), multiple recoveries (range 12-21 per experiment) of individual cells (n=56), five cells batches (n=5) and negative controls (n=8) were carried out. *Ampli*1™ WGA products from the purified cells were DNA-fingerprinted and, along with KRAS sequencing, confirmed cell presence in 91% of single cell recoveries (**Fig.3**). The five tubes with no signal from STR and KRAS analyses suggest that these cells may have been removed with the supernatant before WGA. All successfully amplified cells matched 100% KRAS mutational status and DNA fingerprint (no alleles from the donor WBCs were present in the TCs profile).

Fig.1 Sketch of workflow based on the DEPArray™ Rare Cell Isolation Technology for CTCs separation and molecular characterization from blood samples enriched with Veridex CellSearch® CTC Kit.

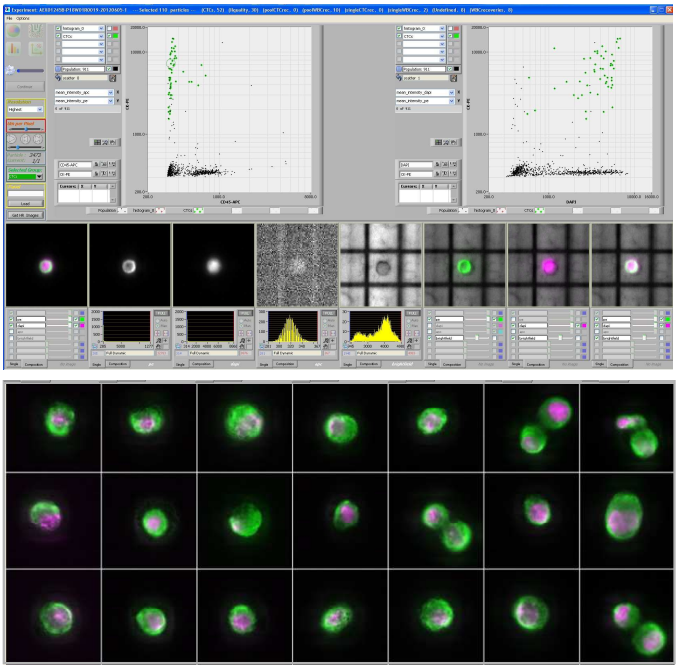

Fig.2 (Top) Screenshot from DEPArray™ system CellBrowser™ with scatterplot of detected cells and an image-bar displaying an individual TC image in Veridex-style (i.e. with DAPI/CK overlay in false colours (magenta/green) and individual channels in greyscale, CK-PE, DAPI, CD45-APC); Bright-Field (BF) is also displayed along with BF/CK, BF/DAPI and BF/CK/DAPI overlay in false colours (grey/green, grey/magenta, grey/green/magenta). (Bottom) Gallery of some TCs detected (DAPI/magenta and CK-PE/green).

DEPArray™ Recoveries	N	KRAS call	KRAS no call	KRAS call rate
Single cells	56	51	5	91%
TC	38	36	2	95%
WBC	18	15	3	83%
5 cells	5	5	0	100%
TC	1	1	0	100%
WBC	4	4	0	100%
Negative Controls	8	0	0	NA

Fig.3 Summary of results of DEPArray™ sorted cells mutational analysis through KRAS sequencing following *Ampli*1™ WGA.

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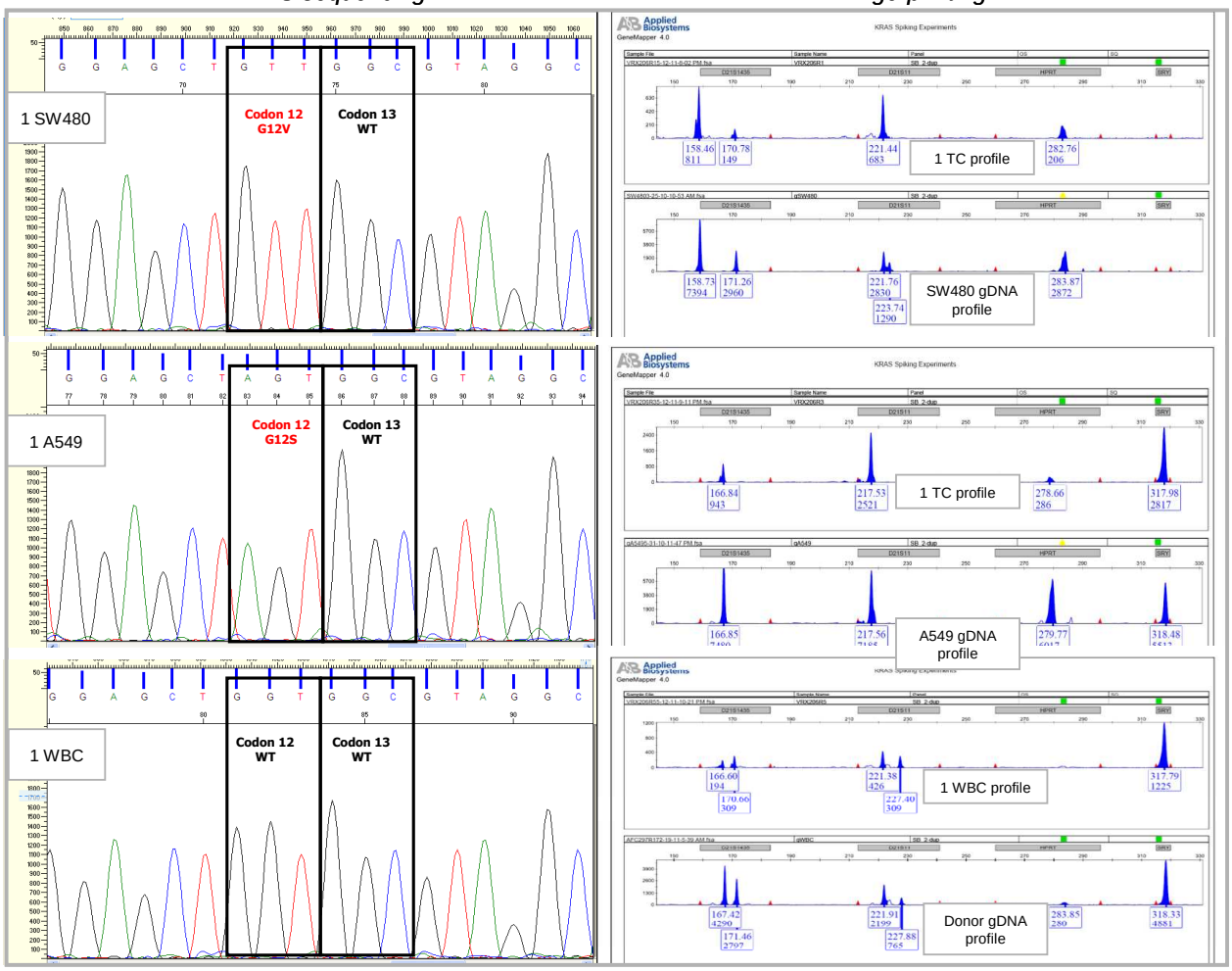

Fig.4 KRAS sequencing and DNA fingerprinting results from cells recovered using a DEPArray™ system from mixed (A549/SW480) TCs spiking in healthy-donor peripheral blood: profile of a single SW480 tumor cell (top), a single A549 tumor cell (middle) and a single WBC (bottom). The DNA fingerprinting demonstrates cells are 100% pure as only the alleles of the relevant cell line or WBC are detected, as further demonstrated by the KRAS mutational profile.

WBCs were correctly wild-type for KRAS and only showed donor's alleles. No signal was detected among NoCell controls recoveries (plain buffer), as expected. Finally, in the mixed tumor cells spiking experiment different specific KRAS mutations and DNA fingerprints were detected in recoveries of different type of TCs (**Fig.4**) reflecting cell heterogeneity, thus clearly detected.

Conclusions

The DEPArray™ platform is shown to enable a highly automated operation for the detection, enumeration and reliable recovery of CTCs thanks to an image-based selection with multiple fluorescent markers and morphology. Furthermore, this new approach using a DEPArray™ system for sorting offers the possibility to isolate a mixed population of TCs downstream of CellSearch® enrichment, with the unique capability to achieve 100%-pure cell recoveries. Such purified cells are thus suitable for molecular characterization with genetic analysis techniques (such as sequencing for mutation analysis) which would otherwise fail due to the large number of contaminant WBCs still present in the enriched samples. Therefore DEPArray™ technology, unlike all other enrichment techniques, allows molecular profiling of pure tumor cells from enriched blood samples and the accurate detection of tumor cell molecular heterogeneity.

References

G Medoro et al, *Use of the DEPArray platform to detect, isolate, and molecularly characterize pure tumor cells from peripheral blood samples enriched using the CellSearch system*, J Clin Oncol 29: 2011 (suppl; abstr 10616), ASCO Annual Meeting, June 2011

Keywords: DEPArray™; CTCs enrichment; CellSearch®; peripheral blood; pure cells; molecular heterogeneity detection

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