Application Note

DEPArray™ Rare Cell Isolation Technology

Isolation of Tumor Cells from Archived Formalin-Fixed Paraffin Embedded Samples

Introduction

Access to the cells contained in tissue samples which are routinely stored as formalin-fixed and paraffin-embedded (FFPE) samples would open the opportunity to obtain valuable comparative genetic information that could result in clinically relevant utility. FFPE tissue samples have been extensively annotated and well preserved, allowing detailed study of the progression of diseases such as cancer. Due to the small amount of tissue available, purifying cells from these samples for downstream characterization has proven difficult. With DEPArray™-based cell isolation and recovery we are now able to address this challenge. Here we investigated the possibility to identify and isolate single and pure tumor and stromal cell from FFPE archived Cervical Carcinoma (CC) patient samples with DEPArray™ technology for DNA analysis.

Materials and Methods

The sample re-suspension and keratin, vimentin, DAPI cell staining procedure for FFPE samples was kindly executed by Dr. Willem Corver according to his published protocol (W.E. Corver and N.T. ter Haar, *Curr Protoc Cytom*. 2011). A small amount of the labeled CC cell suspension, estimated to be a few thousand cells, was loaded into the DEPArray™ cartridge for analysis using Silicon Biosystems' standard procedure. Qualitative and quantitative marker evaluation, along with cell DNA content measurement, was performed with the integrated DEPArray™ instrument analysis software enabling population analysis from scatter plots as well as image evaluation. A much larger fraction of the re-suspended cells was also sorted and analyzed by Dr. Corver's lab using FACS.

Results

The scatter-plot generated by the DEPArray™ instrument clearly shows that it is possible to identify the same populations of stromal, tumor cells and clusters detected by flow cytometry (**fig.1**). The unique features of DEPArray™ for this application are

- the possibility to work with a minute amount of cells (about 3-4 orders of magnitude less than what required by FACS) and

- the capability to visualize the events detected (**fig.2**) and selectively choose for sorting only best and pure cells.

Analysis of tumor cell ploidy status was carried out by comparison with stromal cells using the measurement of integral DAPI fluorescence intensity, linked to total DNA content. The results showed DNA gains in tumor compartment versus the stromal one, as expected (**fig.3**). Finally pure recoveries of tumor and stromal cells were carried out successfully.

Conclusions

Archived FFPE tissues can be used, after suitable sample preparation steps, as starting biological samples for cytometric analysis and pure tumor cells isolation by DEPArray™ technology, overcoming the challenge of dealing with tiny amount of samples. With respect to unsorted samples, purity enables more accurate downstream analysis, improving the sensitivity in the detection of presence/absence of different biomarkers. In large-scale sequencing protocols the possibility offered by DEPArray™ based sorting to selectively isolate "just the cells of interest" offer the opportunity to perform on them a more in-depth genomic analysis as the background of unwanted cells is eliminated, possibly revealing rare mutations among the tumor cell population.

Keywords: DEPArray™; formalin-fixed and paraffin-embedded; cancer; small cell loads; pure cells

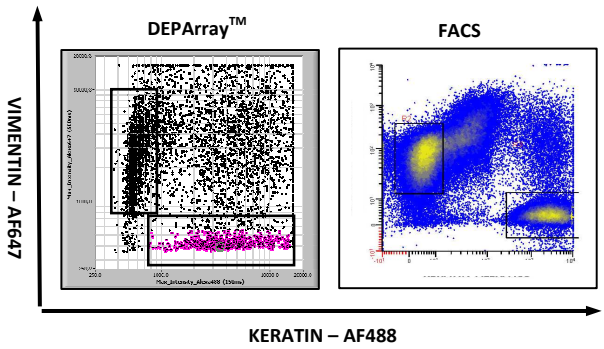

Fig.1 DEPArray™ (left) and FACS (right) scatter plot analysis showed two well-defined populations. The TUMOR cells were on the upper-left side (VIMENTIN$^+$/KERATIN$^-$), whereas the STROMAL cells were on the bottom-right (VIMENTIN$^-$/KERATIN$^+$).

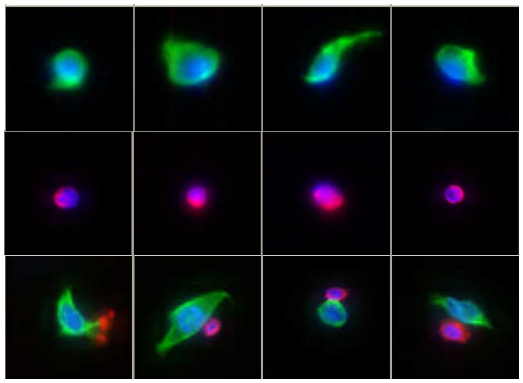

Fig.2 DEPArray™ image galleries of TUMOR cells (KERATIN in green), STROMAL cells (VIMENTIN in red) and clusters of both populations were showed in the first, second and third row, respectively.

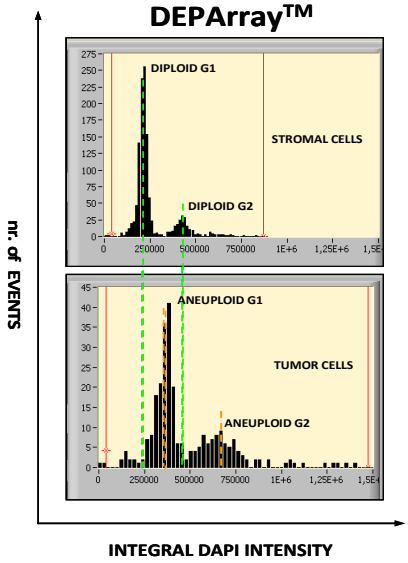

Fig.3 DAPI content of TUMOR and STROMAL cells obtained with DEPArray™ were showed. Aberrant ploidy status of TUMOR cells versus STROMAL cells were easily identified in histograms.

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