DEPArray™ enables recovery of pure tumor cells from heterogeneous fine needle aspirates for routine downstream NGS analysis

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Introduction

In earlier studies, we have demonstrated reliable recovery of pure populations of (rare) cells from complex tissues using the DEPArray™ system. Fine Needle Aspiration (FNA) is often an outpatient and safe procedure routinely used to examine tissue or bodily fluid from a lesion or cyst helping to make a diagnosis or rule out conditions such as cancer. Although FNA is also used to assess the effect of treatment, the procedure may often appear to have failed due to insufficient number of target cells in the sample and contamination with normal cells. In fact, specimens acquired from patients affected by low-tumor-burden disease can be composed of an overwhelming percentage of normal cells. Clinicians hoping to practice precision medicine based on quantifiable molecular characteristics of a tumor specimen must take into account the cellular heterogeneity of the specimen and the inexactitude of the NGS data produced from its DNA. Here we provide preliminary results showing 100% efficiency in recovering pure tumor cell populations from FNA samples of patients affected by metastatic breast cancer and known to have low tumor burden (<20%) prior to using the DEPArray™ platform. Without a precise method of determination, the actual ratio of tumor cells to normal cells in an FNA specimen is unknown.

Method

FNA paraffin embedded sections (50 microns thickness) from metastases originating from breast (n=3) primary tumors were evaluated. Each FFPE curl was processed to yield single cells followed by DEPArray™ sorting based on cytokeratin (Ker), vimentin (Vim) and nuclear staining. The recovered cell populations were directly lysed in the collection tube prior to PCR-based target enrichment for next generation sequencing using Ion AmpliSeq™ CHV2.

Results

DEPArray™ analysis allowed identification of 3 distinct cell populations representing tumor (KER+), stromal (VIM+) and Double Positive (KER+/VIM+) cells. Overall, 21% (4.3% to 42.7% range) of the total [mean of 6335] cells analyzed were of tumor (KER+) origin. Groups of pure cells (mean 105 cells range 15-200) for each population were recovered for sequence analysis. In one of the breast cancer FNA samples analyzed, TP53 loss of heterozygosity is clearly visible in the sorted tumor (KER+) cells and not in the unsorted population. Otherwise, Double Positive (KER+/VIM+) sample shows two copy-number gain of the mutant allele, implying a tumorial trait. In addition, a frequency of somatic heterozygous variant was detected in both tumor and Double Positive populations but notably was absent in stromal cells confirming it as a somatic mutation.

Conclusions

DEPArray™ allows resolution of two main limitations associated with FNA samples obtained for genomic analysis: too few target cells and unwanted admixture of normal cells. DEPArray™ allows for phenotypic distinction between the sorted cells prior to recovery; thus, enabling sequence analysis that is suitable for detecting genomic aberrations such as CNVs and LOH, which cannot be evaluated as precisely in an unsorted sample. Clearly, the DEPArray™ platform brings precision to detection, quantification and recovery of pure target cells that are suitable for subsequent downstream molecular analysis that can improve cancer diagnosis and personalized treatment strategies for breast cancer patients.