Introduction

Tumor cells within biopsies exhibit considerable heterogeneity with multiple sub-populations showing different genetic profiles. Such complexity can result in ambiguous or inconclusive test results and mutations within the tumor cannot be assessed accurately. In addition, the analysis can even fail completely when the tumor cell fraction is underrepresented. Conventional FACS require relative high cell numbers for instrument setup and color controls and the separation is incomplete, producing partially enriched rather than pure, populations of tumor and normal cells. Microdissection is another method for tumor cell enrichment but this approach is inherently labor intensive and purity is compromised by the complexity of tumor cell infiltration patterns. Here we present an innovative workflow combining enzymatic disaggregation and fluorescent staining with DEPArray™ image-based dielectrophoretic cell sorting, which enables isolation of pools of 100% pure tumor cells from tiny clinical samples for subsequent genetic analysis.

Methods

We disaggregated into cell suspensions archival FFPE samples from 1 ovarian, 2 pancreatic, 9 lung, 11 colon cancer paraffin blocks for Vimentin, Keratin and nuclear stain. We sorted by DEPArray™ precise numbers (mean=140, median 94, range=5-600) of pure homogenous pools of cells from the major population of tumor cells, the contaminant diploid stromal cells, and other minority tumor cell type positive for both keratin and vimentin (K+V+). Using IonTorrent AmpliSeq™ CHV2, we generated long sequencing libraries, after direct lysis of the pure cells recovered by DEPArray™, or of the unsorted samples (either QiAmp DNA columns or disaggregated cells). Libraries were sequenced with IonTorrent™ PGM (mean depth>2,000x), and analyzed using Torrent Suite.

Results

On several loci, we detected somatic mutations with 100% variant frequency, only observable as heterozygous in the unsorted samples and as wild-type in stromal cells of same patient, confirming 100% purity of sorted cells. Frequently, for loci harboring germline homozygous SNPs with variant frequency around 50% for pure stromal cells, we readily detected loss-of-heterozygosity in tumor cell sub-populations as binary (0%/100%) variants. Copy number gains were also reproducibly identified in tumor cell replicates as deviations from the 50% variant frequency of germline CNVs of pure stromal cells. Quantitative analyses of a molecular genetic study of intra-tumor heterogeneity, grouping tumors based on their DNA content. Sequencing analyses revealed that homogeneous cell recoveries from different tumor sub-populations might have genetically distinct profiles.

Conclusions

We solved two pressing problems in preparation of FFPE samples for genetic analysis: small sample size and unwanted admixture of normal cells. Regardless of the tumor fraction in input, the procedure leads to robust and reproducible results because i) stromal contaminants are eliminated, ii) the number of input cells is controlled precisely by the instrument. The approach makes possible to reliably analyze another quantitative traits such as CNVs and LOH, which cannot be evaluated precisely in an unsorted sample.

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