Understanding tumor heterogeneity in formalin-fixed paraffin-embedded samples

Introduction

Genomic profiling in solid tumor is notoriously challenging due to the presence of contaminating stromal and other kinds of cells. The analysis can even fail completely when the tumor cell fraction is under-represented. In addition, prevalence of mutations within the tumor cannot be assessed accurately. Microdissection is often attempted to enrich for the tumor cell fraction, but this approach is inherently labor intensive and purity is limited especially when the tumor cells are highly intermingled with stromal cells, e.g. inflammatory cells. An alternative approach to enrich for tumor cells is to dissociate FFPE tumor tissues into cell suspensions and, after staining for cytoskeleton proteins and DNA, attempt a fluorescence-based cytometric analysis using FACS sorters. However, the small sample size, e.g. biopsies, generally available in modern pathology, prevents this approach to be coupled with conventional FACS which require relative high cell numbers for instrument set-up and color controls. Here we present an innovative workflow combining enzymatic FFPE 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 [Polzer 2014].

Methods

Disaggregated into cell suspensions archival FFPE samples from 1 ovarian, 2 pancreatic, 9 lung, 3 adenocarcinoma rectosigmoid, 7 adenocarcinoma rectum, 1 adenocarcinoma sigmoid cancer patient. Cells were stained for Vimentin, Keratin and nuclear stain. We sorted by DEPArray™ precise numbers (mean=133, median 85, 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 types indicative of epithelial-to-mesenchymal transition (EMT).

Using Ion torrent Ampliseq™ CHPv2, we generated NG5 sequencing libraries, after direct lysis of the pure cells recovered by DEPArray™ (n=56), or of the unsorted samples (either QIAPrep DNA columns or dissociated cells). Libraries were sequenced with IonTorrent™ PGM (mean depth=2,000x), and analyzed using Torrent Suite.

Results

On several loci we detected mutations appearing with 100% variant frequency in the replicates of pure tumor cells, variable low level frequency in the unsorted samples and as clean wild-type in the pure stromal cells of the same patient, confirming 100% purity of all sorted cell pools. Moreover, in the EMT-phenotype subpopulations we detected unique putative somatic variants, different from tumor cells majorly and undetectable in unsorted samples. Frequently, for loci harboring germ-line heterozygous SNPs with variant frequency around 50% in pure stromal cells, we readily detected Loss-of-Heterozygosity in tumor cells subpopulations where variant frequencies drop to 0% or jump to 100%. Quantitative traits such as copy number gains and losses were also reproducibly identified with high precision in pure tumor cell replicates, as deviations from the 50% variant frequency of germline SNPs in the pure stromal cells were not justifying a complete loss of one allele but rather an increase in copy number of the same.

Conclusions

We solve two pressing problems in preparation of FFPE samples for genomic 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 it possible to reliably analyze also quantitative traits such as CNVs and LoH, which cannot be evaluated precisely in an unsorted sample.

Highlights

- Sorting tumor pure cell subpopulations reveals their genetic characteristics, undetectable in unsorted samples. Analyzing homogenous cell subpopulations boosts signal-to-noise ratio working around inherent sensitivity/specificity trade-offs of rare-variant calls. Reliable detection of quantitative traits such as CNVs. Sorting pure stromal cells yields internal controls for archival samples.

References

[Polzer 2014] Molecular profiling of single circulating tumor cells with diagnostic intention, Embo Molecular Medicine, September 2014

[Corver 2014] High-Resolution Multiparameter DNA Flow Cytometry for the Detection and Sorting of Tumor and Stromal Subpopulations from Paraffin-Embedded Tissues

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