DEPArray™ digital sorting enables multi-level and multi-resolution genomic profiling of FFPE samples with low tumor content

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Introduction

Deciphering the complexity of tumor populations represents a great challenge for precision medicine, enabling the cancer patients to receive personalized therapies. Poor tissue biopsies and low-tumor content hinder this goal. Here we describe how DEPArray™ sorting technology enables a complete tumor characterization in pure populations or single cells of FFPE samples at low tumor cellularity.

Methods

Two FFPE 5µm thick sections from pancreas ductal adenocarcinoma, with <30% tumor content, were processed by DEPArray™ sorting protocol enabling cell pools and single cells from pure stromal and tumoral populations (Fig. 1.2). For Whole Exome Sequencing (WES), stromal (n=142) and tumor (n=128) cells were recovered and lysed. Illumina-compatible libraries were prepared, enriched using Agilent SureSelect capture kit and sequenced on HiSeq 4000. For copy number analysis (CNA) analysis, low-pass Whole Genome Sequencing (WGS) on MiSeq was performed with a pre-capture aliquot of WES libraries in addition to a library obtained by unsorted cells (gDNA). Low-pass WGS was performed also on tumor (n=6) and stromal (n=5) tissue-derived single-cells, after a whole-genome amplification step performed using Ampli1™ WGA kit. Moreover, other cell pools from stromal (n=203) and pure tumor (n=122) populations were used as input in an amplicon-based assay, using DEPArray™ Oncoseek Panel with the aim of cross-validating results obtained with different approaches.

Results and discussion

WES analyses identified several non-synonymous somatic mutations relevant in pancreatic cancer, such as KRAS:p.G12D, SMAD4:p.R361H, TP53:p.R273H and CDKN2A:p.R80* variants (Fig. 3). Furthermore, all mutations covered by DEPArray™ Oncoseek panel were confirmed. In sorted pure-tumor populations, a combined analysis of low-pass CNA profiles and WES B-allele frequency (BAF) plots clearly identified several Loss-of-Heterozygositity (LOH) as well as absolute copy-number alterations (Fig. 3a), which include many clinically relevant genes. On the other hand, the profile obtained by stromal population is flat as expected. Same fine tumor characterization of gains and losses is not detected in the unsorted population, due to a "dilution" effect determined by the low-tumor cellularity. Interestingly, an homozygous loss was detected and confirmed by three independent methods, which consist of CNA calling by low-pass WGS and WES, and BAF profiles obtained by WES data. This homozygous loss is located on chromosome 9, in a small genome segment including PTPRD onco-suppressor gene (Fig. 4d).

Although showing a high level of similarity with pool of cells, single-cell copy-number profiles revealed several level of inter-cell heterogeneity, showing private aberrant regions. For the purpose, a titration test was conducted measuring the consistency of CNA profiles employing pools with different number of cells, ranging from 117 down to single-cells. A ROC analysis, performed using the un-amplified genomic DNA from sorted tumor population as reference, resulted in a mean Area Under Curve (AUC) = 0.93, with lower AUCs (0.87) observed by single-cells (Fig. 5).

Conclusions

DEPArray™ sorting technology represents a necessary tool for investigating the cancer genome complexity from low-cellularity FFPE tumor samples, demonstrating an unambiguous and unprecedented description of tumor-specific variants and alterations both at population and single-cell level.