Abstract #326

Introduction In the era of targeted therapy, decoding intra-tumor heterogeneity is of critical importance, since hidden genetic features can guide the decision-making process. For this reason, analyzing only one type of biological specimen may provide only partial information. Here we report a multi-level approach to analyze circulating tumor cells (CTCs) and formalin-fixed paraffin-embedded (FFPE) tumor tissue-derived cells (TCs) obtained from the same patient, to investigate the underlying tumor genetic heterogeneity.

Methods Specimens from two advanced lung adenocarcinoma patients were analyzed. The first patient, carrying an ALK translocation, was treated with cisplatin-pemetrexed as neoadjuvant therapy and with an ALK-inhibitor; the second patient was treated with carboplatin-pemetrexed. Blood samples were enriched either with an EpCAM-based or EpCAM-independent method, and matched archival FFPE sections were obtained from pleural effusion cell blocks and primary tumor tissue respectively. The enriched blood samples were stained with Cytokeratin-PE, CD45-APC and DAPI while, after dissociation, cells from tissue were stained with Vimentin-APC, Keratin-FTC and DAPI. DEPArray™ platform detected and recovered single pure CTCs or TCs, along with WBCs or stromal cells as controls. Single-cells DNA was amplified with Amp1™ WGA kit and used to obtain genome-wide copy-number alterations (CNA) profiles using the Amp1™ LowPass kit. Aliquots of FFPE tumor and stromal cell lysates were used as input for the DEPArray™ OncoSeek targeted panel.

Results Low-pass copy-number profiles of both FFPE single-cells and CTCs from the first patient revealed an overabundance of gains and losses, confirming the aberrant nature of tumor cells. All single cells showed a large pattern of shared alterations, with a common amplification of a genome region comprising MYC gene, and some minor differences which indicate intratumor heterogeneity. A hierarchical unsupervised clustering clearly separates WBCs, described by a flat profile, from the group of FFPE single-cells and CTCs, with the latter characterized by a wider inter-cell heterogeneity. Noteworthy, copy-number analyses obtained with OncoSeek panel confirmed a 3-fold amplification of MYC gene. The analysis of the second patient resulted in a quite different situation, where clustering of low-pass profiles highlighted an independent group formed by FFPE single-cells clearly distinct from the highly heterogeneous cluster formed by CTCs.

Discussion DEPArray™ technology provides a reliable approach to digitally isolate 100% pure single tumor cells from different sample types from the same cancer patient. The precision granted by this platform allows to readily dissecting genetic intra-tumor heterogeneity and the evolutionary mechanisms underlying carcinogenesis.

Patient 1: DEPArray™ Image gallery and LPCNA analysis of single CTCs/TCs

Patient 2: DEPArray™ Image gallery and LPCNA analysis of CTCs/TCs

Figure 1. Scatter plots of FFPE (A) and blood-derived enriched samples (B) with relative image galleries of Tumor Cells (B) and CTCs (D), LPCNA profiles of TCs (C) and CTCs (F) highlight a gain on an area of the chromosome 8 corresponding to MYC locus (in blue). G: hierarchical clustering of CTCs and TCs reveals different subpopulations of cells. Histograms depicted in (H) and (I) quantify MYC amplification observed both in CTCs and in TCs respectively.

Figure 2. Scatter plots of FFPE (A) and blood-derived enriched samples (C) with relative image galleries of Tumor Cells and CTCs; LPCNA profiles of TCs (B) and CTCs (F) confirm the aberrant nature of the cells, while the hierarchical clustering of CTCs and TCs in (E) shows a clear separation of the two cell populations.

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