Molecular characterization with single-cell resolution of CTCs and FFPE specimens from the same lung adenocarcinoma patients reveals the extent of intra-tumor heterogeneity

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Background  Intra-tumor heterogeneity can hide genomic and genetic features, which may be key drivers of disease progression. Routinely, only one biological specimen per patient is generally analyzed, which may only partially represent the genetics of the tumor. Here we report a multi-approach analysis of circulating tumor cells (CTCs) and formalin-fixed paraffin-embedded (FFPE) tumor tissue-derived cells (TCs) obtained from the same patients, to investigate the underlying genetic heterogeneity.

Methods  Peripheral blood (PB) and FFPE tumor tissue were collected from two advanced lung adenocarcinoma patients, treated with cisplatin-pemetrexed and carboplatin-pemetrexed respectively as first line therapy. The first patient was previously diagnosed an ALK- translocation and treated with an ALK-inhibitor. PB was enriched with either an EpCAM-based or EpCAM-independent method: the cell output of the latter was stained with Cytokeratin/CD45-APC and DAPI. Matched 50 µM thick FFPE sections were obtained from pleural effusion cell blocks for the first patient or from primary tumor tissue for the second one; after dissociation, cells were stained with Vimentin-APC, Keratin-FITC and DAPI. The DEPArray™ platform was used to detect and collect single CTCs or TCs, along with WBCs or stromal cells as controls. Whole genome amplified DNA of single CTCs and TCs was used to profile genome-wide copy number aberrations (CNAs) with the Ampli1™ LowPass kit; single nucleotide variants were analyzed on CTCs WGA products and on pools of TCs using Ampli1™ CHP custom panel and DEPArray™ OncoSeek panel respectively.

Results  No clinically significant variants were detected in CTCs and FFPE samples; however the copy-number profiles of single TCs and CTCs revealed an overabundance of gains and losses, confirming the aberrant nature of tumor cells. In the first patient, all single cells showed a pattern of shared alterations, with a common amplification of the genome region comprising MYC gene (also confirmed by depth-of-coverage in targeted panel). A hierarchical unsupervised clustering clearly separated WBCs, from the group of TCs and CTCs, characterized by some emerging clones and low inter-cell heterogeneity. The analysis of the copy-number profiles of cells from the second patient showed an opposite situation: unsupervised clustering of low-pass profiles highlighted an independent group formed by single TCs clearly distinct from the highly heterogeneous cluster formed by CTCs.

Discussion  The precision granted by analysis of pure cells derived from multiple specimens from the same patient, together with the combination of low-pass whole-genome sequencing and targeted sequencing, reveals unexpected genetic similarities and diversities, and provides fundamental information to understand intra-tumor heterogeneity.