Digital sorting and single-cell genomic profile comparison of lung adenocarcinoma CTCs between EpCAM and size-based enrichment methods

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Background Several methods are currently available for Circulating Tumor Cells (CTCs) enrichment. Many studies compared CTC counts between FDA-approved CellSearch® system (based on EpCAM enrichment) and size-based selection methods. However, little is known about genomic differences across CTCs obtained from different platforms. For the first time, we report here a genomic characterization of copy-number and sequence variants in single CTCs enriched with different methods from the same patient.

Methods Peripheral blood (PB) was collected from an advanced lung adenocarcinoma patient treated with cisplatin-pemetrexed-necitumumab as first line therapy and with carboplatin-gentamicin as second line therapy. Two PB samples were collected at the same time: one was enriched with CellSearch® System, and the other one with Parsortix® (size-based selection), followed by Cytokeratin-APC, CD45-PerCP Cy5.5 and DAPI staining. Both samples were analyzed with DEPArray™ system and single CTCs along with single WBCs as controls, were isolated. Recovered cells were whole genome amplified with Ampli1™ WGA Kit and their Genome Integrity Index (GII) was assessed. On IonTorrent™ PGM Platform, we profiled Genome-wide copy-number alterations (CNA) by low-pass whole-genome sequencing with Ampli1™ LowPass Kit, and analyzed cancer-gene sequence variants with Ampli1™ CHP Custom Beta targeted panel (Figure 1.)

Results Single CTCs (CK+, CD45-, DAPI), showing GII ≥ 3, either isolated from CellSearch®-enriched or PR1-enriched blood, along with WBC, were selected for NGS. Ampli1™ LowPass Kit data showed several aberrations confirming tumor origin of all CTCs, while WBCs (n=6) produced balanced profiles (Figure 3). Unsupervised hierarchical clustering segregated PR1-derived (n=6) from CellSearch®-derived (n=4) single CTCs in two separate branches. Parsortix®-derived CTCs were very similar to each other, with the exception of only one CTC that clustered together with those enriched with CellSearch®. CellSearch®-derived CTCs showed comparable level of heterogeneity and similarity. Some aberrations were common to all CTCs across the two platforms (Figure 2). At the sequence level, the targeted panel revealed somatic mutations shared by all CTCs (FLT3), a PTPN11 subclonal mutation (in 3/6 Parsortix and 2/4 CellSearch), and other private mutations in single CTCs.

Discussion The combination of low-pass whole-genome sequencing and targeted sequencing on single CTCs sorted from PB enriched with different platforms, revealed genetic similarities and diversities. Although this evaluation is here limited to one patient, interestingly the CNAs profiles clustered in two different CTCs populations which matched the two enrichment methods, suggesting a possible correlation between cells phenotype and genotytype. On the other hand the mutations analysis revealed a more heterogeneous situation, confirming the overall CTCs heterogeneity.

Figure 1. Parsortix® System

Figure 2. DEPArray™ Image gallery of CTCs and LPCNA analysis

Figure 3. Panel reporting the overall results of the study: from right to left it is shown, for each CTC, the relative image gallery, LPCNA and the frequency of point mutations (0%, 50% and 100%).

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