The genetic heterogeneity of Circulating Tumor Cells: a longitudinal study in breast cancer patients

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Background A relevant percentage of breast cancer patients relapse with metastatic disease, whose genetic understanding is still fragmentary. Metastatic disease is initiated by circulating tumor cells (CTCs) that originate from the primary tumor and spread in the blood circulatory system. CTCs analysis represents today a reliable and non-invasive liquid biopsy for the detection of tumor genetic heterogeneity, despite difficulties in defining their characteristics due to their substantial pleomorphism. Here we performed genetic characterization on pure single CTCs isolated from metastatic breast cancer patients, aiming to provide a full genetic picture of multiple single CTCs for each patient.

Methods Peripheral blood of 4 de novo diagnosed metastatic breast cancer patients, ER+/HER2−, treated either with hormonal therapy or with weekly paclitaxel/gemcitabine and paclitaxel/bevacizumab as first line therapy, was collected at different time points (before start, after one cycle of treatment and at tumor progression) (Table 1). CTCs were enriched with the CellSearch® system and individually sorted with DEPArray™ platform. The DNA of each single CTC was amplified with Ampli1™ WGA kit and Genome Integrity Index (GII) assessed by Ampli1™ QC kit. WGA products were used for genome-wide single cell copy number variation (CNV) analysis with Agilent SurePrint 180k array for comparative genomic hybridization (aCGH). Furthermore, Ampli1™ WGA products were also sequenced with Ampli1™ CHP Custom Panel on Ion Torrent PGM, at 100X average coverage on Ion 316 or 318 chip (Figure 1).

Results A total of 100 single CTCs were collected and 69 (69%) showed high Genome Integrity Index (GII) as measured by Ampli1™ QC kit (GII ≥ 3). For each time point multiple single CTCs were selected for single cell aCGH and processed with Ampli1™ CHP Custom Panel Beta for detection of over 2200 COSMIC mutations across 49 genes. Aberrant karyotype of CK+/CD45−/DAPI+ CTCs, as assessed by the aCGH analysis, confirmed the tumoral origin of the cells. Furthermore the additional antibody against cancer stem cell marker CD44, used for the staining with the Celltracks®, identified CK+/DAPI+/CD45+/CD44+ cells which also showed a tumoral genetic profile. In 3 out of 4 patients (3, 12 and 13), aCGH analysis pointed out changes in the CNV profile at different time points, as well as differences between profiles among cells from the same time point. By contrast, patient 7 had all CTCs with a strikingly similar CNV profile both among cells isolated at the same time point and longitudinally. Ampli1™ CHP Custom Panel Beta revealed several COSMIC mutations in coding sequence of different genes, among which frequent TP53 mutations which were further confirmed by Sanger sequencing. Interestingly patients #6 and #12, further to CD44 expression, showed also mutations of AKT1 and PIK3CA genes respectively, which is a documented condition associated to Epithelial to Mesenchymal Transition mechanism (EMT) and in general to an increased malignancy of breast cancer cells. When present, mutations in PIK3CA and AKT1, were comparatively more pervasive, between CTCs at the same time point, and persistent longitudinally.

Conclusions Breast Cancer CTCs display different types and degrees of heterogeneity in genomic aberrations both at the level of CNVs and Sequence variants. The preliminary results from this longitudinal study show clear evidence of dynamic changes in the genome as well the possibility for stable genomic CNV profile across time points and between cells. Further investigation is warranted to correlate the impact of this heterogeneity and dynamics in relation to treatment selection and patient clinical management.

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