Longitudinal genetic characterization of Circulating Tumor Cells in metastatic breast cancer patients

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Background The high heterogeneity of metastasis and the lack of effective treatments capable to counteract their spreading, make the metastatic lesions the major cause of cancer-related death. Little is known about the evolution of genetic aberrations during metastatic cancer progression and in response to systemic treatment. Obtaining repeated tissue biopsies is often impossible but the presence of circulating tumor cells (CTCs) in patients' peripheral blood correlates with the metastatic progression. Recently it has also been shown that CTCs can be easily followed during disease course and isolated at the single cell level to be further processed for genetic characterization with high reliability.

Methods Peripheral blood from 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). The CTCs enrichment was performed with the CellSearch® system and from each sample individual CTCs were sorted with DEPAArray™ Platform. The DNA of each single CTCs was amplified with AmpT1™ WGA kit and the Genome Integrity Index (GII) was assessed by AmpT1™ QC kit. Each AmpT1™ WGA output was used for genome-wide single cell copy number variation (CNV) analysis with Agilent SurePrint 180k array for comparative genomic hybridization (aCGH). Furthermore AmpT1™ WGA products were also sequenced with AmpT1™ CHP Custom Panel on the Ion Torrent PGM, at 1000x average coverage on Ion S16 or 318 chip (Figure 1).

Table 1: Patient data overview with the tumor characteristics, the type of treatment and the relative drug. For each time point the CellSearch® CTCs count and the number of AmpT1™ WGA products analyzed with aCGH and NGS2 have been reported. Patient #7 presents a further lobular collection (72 assent sampling), which has been done between the 1st cycle of treatment and the tumor progression time point.

Results A total of 100 single CTCs were collected and 69 (69%) showed high Genome Integrity Index (GII) as measured by AmpT1™ QC kit (GII ≥ 3). For each time point multiple single CTCs were selected for single cell aCGH and were sorted with AmpT1™ CHP Custom Panel Beta. As expected, malignant origin of CK+/CD45−/CD44+ CTCs was confirmed, as assessed by the CNV profile with the time aCGH approach.

Interestingly, the additional antibody against cancer stem cell marker CD44, used for the staining with the Celltrack®, identified CK+ /CD45+ /CD44+ cells which also showed a tumor genetic profile. The AmpT1™ CHP Custom Panel Beta unraveled a significative number of point mutations of different genes coding sequence, which were further confirmed by the Sanger methodology. All sequence variants for which Sanger sequencing was completed were concordant, in terms of genotype and variant frequency.

Discussion In 3 out of 4 patients (8, 12 and 13), aCGH analysis pointed out a global change in terms of genetic aberrations across the different time points, in particular for patient #8. For each cell a complete genetic picture was created, highlighting also for patient #7 a great genetic heterogeneity among cells showing the same phenotype and also within the same point (Figure 2).

Figure 1: Sample workflow

Figure 2: Result overview displaying, from right to left, the cells image gallery (aCGH), the aCGH overview images, the aCGH profile and point mutations observed on different genes together with the relative DEPArray™ profile for each time point. A gene list box on the right describes the frequency (0-100%) thus a blank box indicates that mutation is wild-type. In line-covered position, where there is a lack of data for variant calling, the box is grey.

DEArray™ Image gallery of CTCs and aCGH analysis

Patient 7

Patient 8

Patient 12

Patient 13

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