Comparison of gene alterations for HER2 positive single circulating tumor cell (CTC) and autologous leukocytes in metastatic breast cancer (MBC) by single cell exome sequencing

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**Introduction**: The monitoring of CTCs in MBC showed ability to predict treatment resistance and outcome. Overexpression of HER2 protein has been associated with rapid cell division and prognosis of advanced breast cancer. Herein, we report a novel finding of specific gene alterations in HER2+ CTC compared to autologous leukocytes in MBC patients.

**Methods**: Whole blood sample (7.5ml/each) was collected from stage III/IV MBC patient before therapy. CTC enumeration was performed by CellSearch™ System using staining for CK, DAPI, CD45 and HER2. The single HER2+ CTC and leukocytes were isolated by DEPAarray™ System (Menarini). Single cell DNA and initial library was prepared by using Ampli1™ WGA and AMPure XP kit (Beckman). The exome capture was performed by TruSeq DNA Exome kit and the sequencing was performed on the NextSeq 500 (Illumina).

**Results**: We identified 107 CTCs by CellSearch™, including 55 HER2+ CTCs and 14 CTC-clusters. Autologous single HER2+ CTC (HER2' CK' CD45', Group 1), HER2' CK CD45' leukocyte (Group 2) and HER2 CK CD45' leukocyte (Group 3) were sequenced respectively. The sequencing data was processed following the GATK pipeline and annotated using SnpEff. There were 486,119 counts (56.69%) for intron variants, 175,819 counts (20.51%) for intergenic variants, 70,334 counts (8.20%) for exon variants, 50,370 counts (5.87%) for downstream genes, 45,915 counts (5.36%) for upstream genes and others (3.37%) in HER2+ CTC. Meanwhile, there were 71,848 (8.76%) and 0 counts for exon variants found in Group 2 and Group 3 respectively. There were 79 gene variants (SNP and Ins-Del) identified to have the highest impact effect (≥20) on HER2+ CTC chromosome, when there were 85 and 0 highest impact gene variants were identified in Group 2 and Group 3 respectively. Among the top 50 high impact gene variants, there were 26 genes alteration sites were same in Group 1 and Group 2, including FECH, HDAC8, CYP11B2, TTN(6 sites), RAN, CD207, HK1, CASP1(2), BRCA1, PIK3CG (3), APEX1(4), KIF11, SIRT5 (2), XYLB and CHKA. In compare to Group 2 the specific gene alterations in Group 1 include CCNA2, FOLH1, BRD4, SAMHD1, CYP17A1, IDE, HPGDS and CTNNB1.

**Conclusion**: Genomic characterization of HER2+ CTC compared to autologous leukocytes elucidated new specific gene alterations associated with disease metastasis in MBC, which will help to develop novel drugs aimed at the eradication of CTCs using molecularly driven therapies for disease metastasis. Furthermore, the new found HER2'CK-CD45+ cells with gene alterations maybe a new kind of cancer transformed leukocytes which need further validation.