Mass cytometric detection of homologous recombination proficiency in circulating tumor cells to predict chemoresistance of metastatic breast cancer patients

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Abstract

Background: Circulating tumor cells (CTCs) can serve as a liquid biopsy to gain insight into treatment responses and metastatic recurrence. Due to their rarity, analysis of CTCs is challenging and commonly based on immunomagnetic technologies using antibodies against EpCAM. This study used mass cytometry (CyTOF®) for identification and characterization of CTCs from longitudinally monitored metastatic breast cancer (mBC) patients. Functional analysis focused on DNA damage responses, particularly the DNA repair pathway of homologous recombination (HR).

Objective: This study aims to evaluate the feasibility of CyTOF® for unbiased detection and characterization of CTCs in mBC patients. Specifically, it compares CyTOF®-based identification of CTCs with CellSearch® and ctDNA-based approaches, assesses CTC-specific phenotypes related to DDR markers (RAD51 and γH2AX), and determines the functional HR deficiency (HRD) status of CTCs to enhance real-time monitoring of treatment responses and chemoresistance.

Methods: Fifty-two blood samples from 13 mBC patients were analyzed for CTC enumeration using CellSearch®, CyTOF®, and ctDNA shallow whole genome sequencing (sWGS). A 13-antibody panel was applied in CyTOF® to phenotype CTCs, including DDR markers (γH2AX, RAD51, 53BP1) and epithelial-mesenchymal transition (EMT) markers. Functional HRD status was inferred based on γH2AX and RAD51 expression in CTCs and compared to genomic HRD detected in ctDNA.

Results: CyTOF® identified CTCs with high sensitivity, correlating significantly with CellSearch® and ctDNA-based tumor fraction quantifications. CTCs exhibited dynamic DDR responses under genotoxic therapy, with γH2AX expression increasing significantly during treatment. HRD status, as determined by CyTOF®, showed dynamic changes over treatment courses, indicating therapy-induced alterations in DNA repair pathway usage.

Conclusion: CyTOF®-based phenotyping provides a robust method for real-time monitoring of CTCs, offering insights into DDR and HR status to predict chemoresistance in mBC patients. This approach could refine treatment strategies by enabling functional assessment of tumor evolution during systemic therapy.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.