HER2 Status Profiling on Isolated Circulating Tumor Cell on the MiSelect R System

Background

The spread of tumor cells into blood circulation plays a key role in cancer progression. These circulating tumor cells (CTCs) provide cellular information such as biomarker expression. CTC phenotype determination might be useful in predicting the efficacy of targeted therapy or immunotherapy. Therefore, CTCs and biomarker characterization may offer an attractive approach for non-invasive monitoring of the expression of therapeutic targets in cancer patients.

The MiSelect R System (MiCareo Taiwan Co., Ltd.) for rare cell isolation and identification can be applied to CTCs for biomarker assessment and enumeration. Here, we demonstrate the capabilities of the MiSelect R System for HER2 expression in breast cancer patients.

Methods

1. Sample Preparation

Blood samples are collected into EDTA-coated vacutainer tubes and processed within 48 hours. The first tube is discarded to reduce contamination of samples with skin epithelial cells. For spiked assay, a predetermined number of cultured tumor cells is added into blood drawn from healthy subjects. The sample preparation is shown in figure 1 below.

Briefly, two 4 mL aliquots of blood are transferred from K2EDTA tubes into 50 mL conical centrifuge tubes. Whole blood samples are incubated with EpCAM for 20 minutes on a rocker. After staining, each blood sample is diluted with Isoton and then gently centrifuged to remove the free antibody. The blood is then processed using the MiSelect R System within 30 minutes of sample preparation.

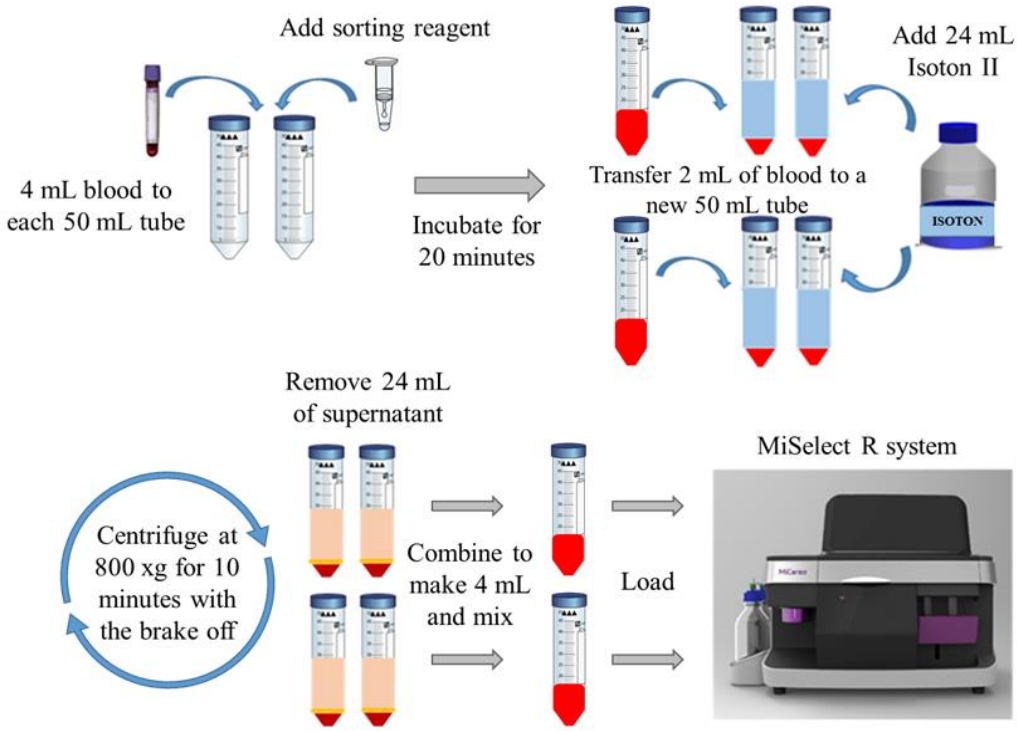


Figure1. Whole blood sample preparation

2. CTC identification with the MiSelect R

The MiSelect R System automatically labels and images the isolated cells with a panel of fluorescent antibodies for phenotypic profiling. A cell is classified as a CTC when its morphological features are consistent with that of a tumor cell and it exhibits the phenotype EpCAM(+), CK(+), DAPI(+), and CD45(-). The CTC number is counted, analyzed, and recorded.

3. HER2 status interpretation on isolated CTCs

HER2 protein expressions are examined with immunofluorescent staining. The mean fluorescent intensity of each isolated CTC is calculated using cell analysis software. The HER2 status of patients is determined by the pathological guidance for HER2 IHC scoring. HER2-positive status is defined as a score of 3+. A 2+ scoring is equivocal, and a HER2-negative status is defined as a score of 0 or 1+. A score of 0 means no observable staining or incomplete and faint staining in ≤ 10% of the cells. A score of 1+ is incomplete faint membrane staining in > 10% of cells. A 2+ score is incomplete or weak to moderate circumferential membrane staining in > 10% of cells; or complete and circumferential membrane staining that is intense and in ≤ 10% of cells. A score of 3+ is intense circumferential membranous staining in > 10% of cells.

Results

1. Quantitative analysis of HER2 expressions in breast cancer cell lines

SKBR3, MDA-MB-361, BT-20 and MDA-MB-231 cells, which are represented as HER2 3+, 2+, 1+, and 0 respectively, are spiked into blood samples from healthy subjects and run on the MiSelect R System. The acquired images (Figure 2) show the outcome for each cell line.

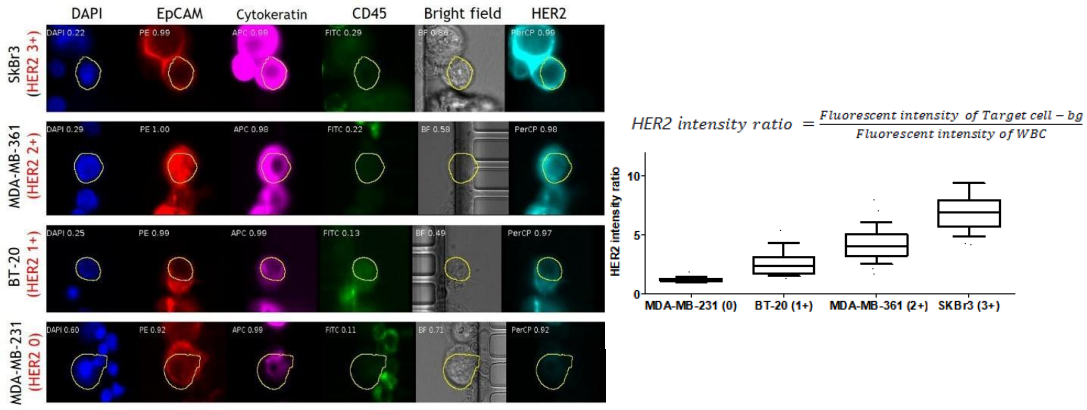
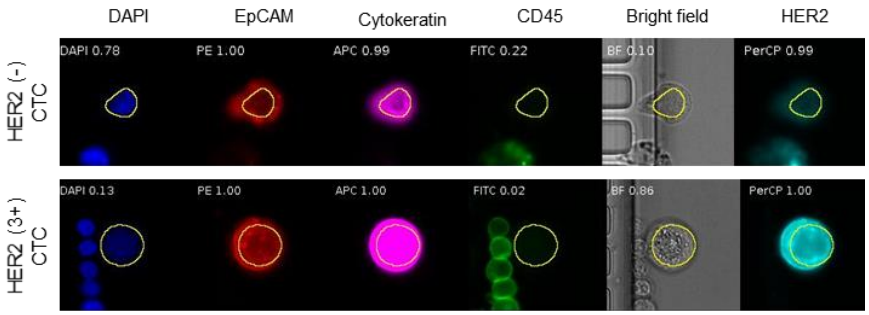


Figure 2. HER2 expressions in different cell lines.

2. HER2 expression on isolated CTCs from metastatic breast cancer patients

Blood samples from 9 metastatic breast cancer patients are used to perform CTC enumeration. Each isolated CTC is examined for HER2 expressions. HER2 expressions show heterogeneity among CTCs within and between patients (Figure 3). The HER2 status of the CTCs shows concordance with pathological evaluation (data not shown).



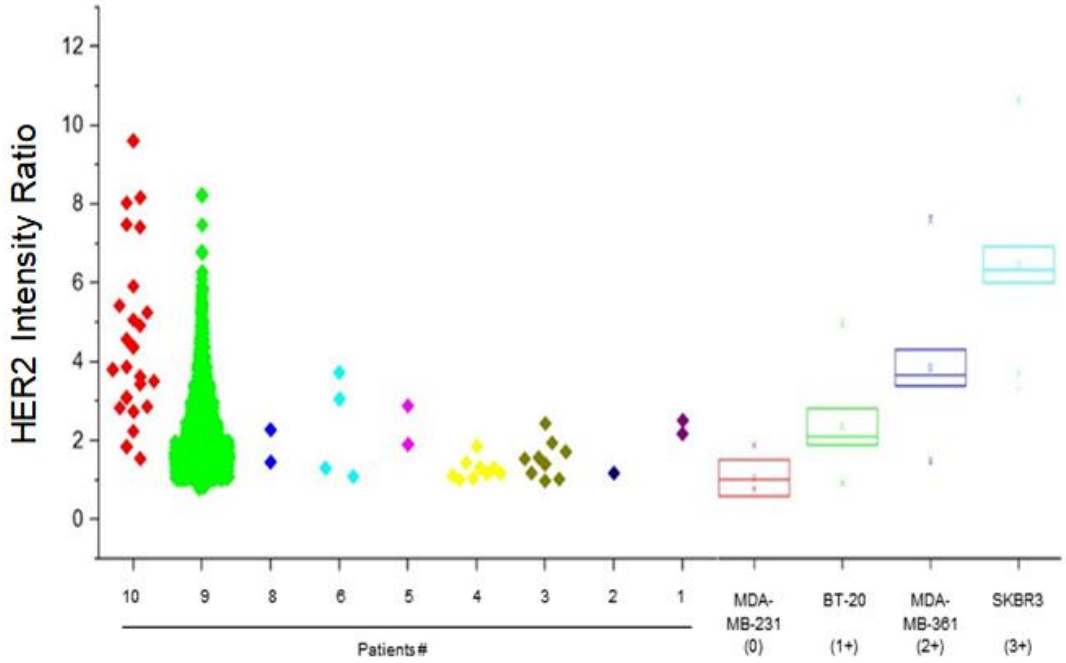


Figure 3. HER2 intensity ratio of each isolated CTC in metastatic breast cancer patients

Conclusion

The MiSelect R System is able to differentiate HER2 expression on isolated CTCs and the expression of HER2 on CTCs are heterogeneous within a single patient sample and between different patients.