PD-L1 Expression Analysis of Isolated Circulating Tumor Cells on the MiSelect R

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.

Modulating immune inhibitory pathways is a major breakthrough in cancer treatment. Checkpoint blockade antibodies targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell-death protein 1 (PD-1) demonstrate acceptable toxicity, promising clinical responses, durable disease control, and improved survival in some patients with advanced melanoma, non-small cell lung cancer, head and neck cancer, and other cancers.

PD-L1 IHC using 22C3 antibody is the only FDA-approved companion diagnostic for selecting NSCLC patients for pembrolizumab. There are many complicating variables in these IHC assays. For example, tumor cells, immune cells, as well as stroma cells can express PD-L1 with considerable heterogeneity within the tumor microenvironment, and PD-L1 expression in primary tumor is induced by IFNγ during disease progression and treatment.

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 PD-L1 expression in various types of cancers.

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.

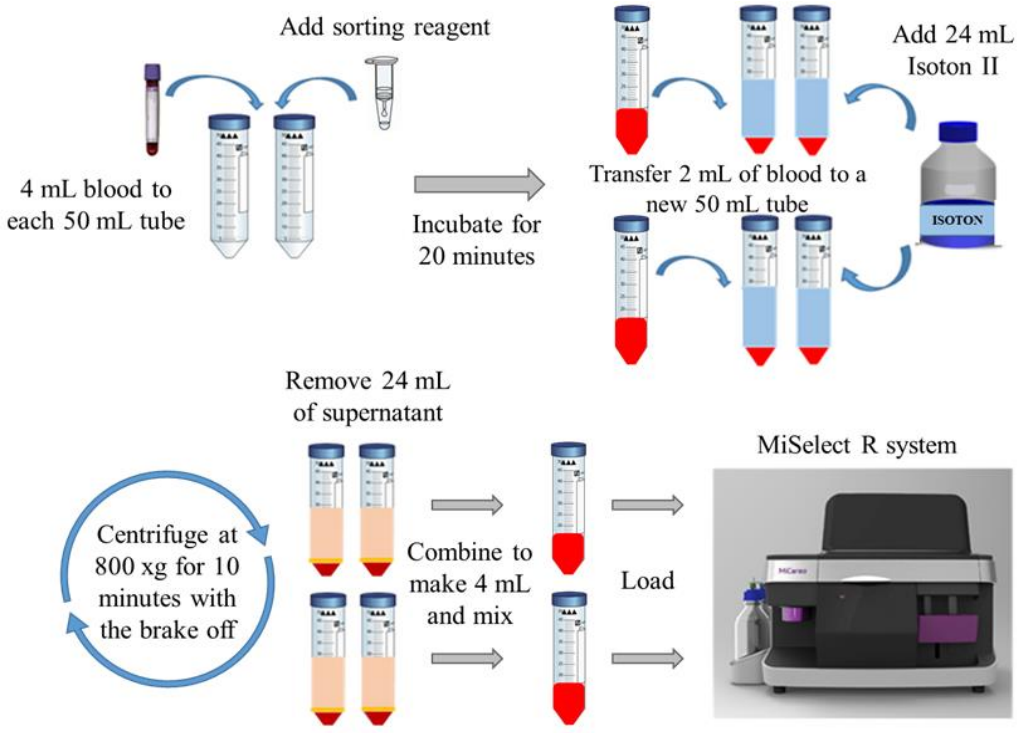

Figure1. Whole blood sample preparation

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. PD-L1 expression on isolated CTC

PD-L1 protein expressions are examined with immunofluorescent staining. The mean fluorescent intensity of each isolated CTC is calculated using cell analysis

software.

Results

1. Quantitative analysis of PD-L1 expression in breast cancer cell lines

H1975, A549 and H441 lung cancer cells were spiked into blood samples from healthy subjects and run on the MiSelect R System. The acquired images (Figure 2) show the expected outcome, with the H441 cells strongly expressing PD-L1, and the A549 and H1975 cells displaying low expression of PD-L1.

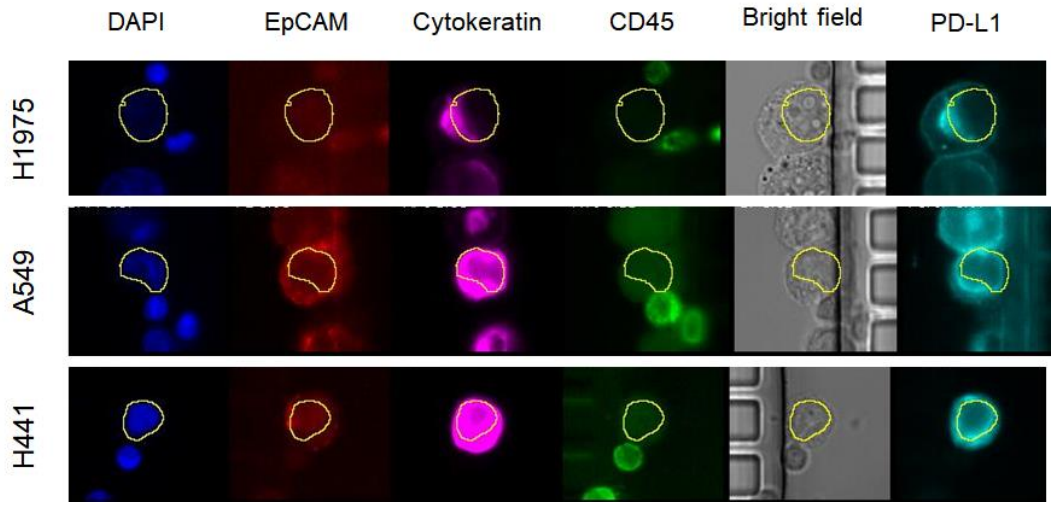

Figure 2. PD-L1 expression in H1975, A549 and H441 cell lines.

2. PD-L1 expressions on isolated CTC from cancer patients

Blood samples from colorectal cancer (CRC) and head and neck cancer patients are used to perform CTC enumeration and PD-L1 expression analysis. PD-L1 expression show heterogeneity among CTCs within and between patients (Figure 3).

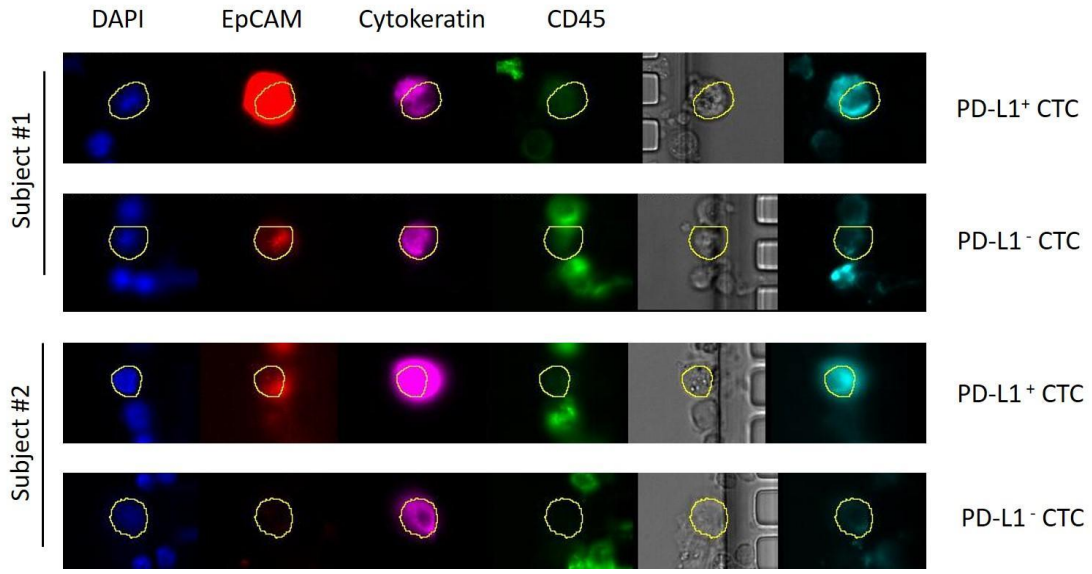

Figure 3. PD-L1 expressions on CTCs in CRC and head and neck cancer patients.

Conclusion

The MiSelect R System shows excellent capability in single cell analysis of rare cells from whole blood as well as versatile capability for multiple-biomarker characterization. It potentially provides important cellular and molecular information at the single-cell level.