Abstract

Recent studies indicate that patients with solid tumor cancers, including colorectal cancer, who harbor genetic defects in DNA mismatch repair will respond to monoclonal antibody based immunotherapy, such as programmed cell death protein 1 (PD-1) inhibitors. Mismatch mutations of microsatellites, called microsatellite instability (MSI), are caused by the sporadic deficiency of mismatch repair proteins (dMMR), which often is associated with colorectal cancer, breast cancer, thyroid cancer, melanoma, endometrial cancer, gastrointestinal cancers, germiinary cancers, and others. Currently, dMMR is diagnosed by staining patient tissue proteins MLH1, PMS2, MSH2 and MSH6 by immunohistochemistry (IHC). We developed a non-invasive liquid biopsy test which enables physicians to measure the dMMR status in circulating tumor cells (CTCs) from patient blood. CTCs in blood are labeled with a tumor specific antibody capture cocktail followed by CTC enrichment in a microfluidic device and stained for MMR proteins. dMMR status on CTCs is determined by automated microscopy.

Methods

MMR protein expression levels on carcinoma cell lines were identified by flow cytometry. For analytical validation, HT29, LoVo, and HCT15 cells were spiked into whole blood using CEESure™ blood collection tubes. Samples were prepared in replicate, on different days, incubated overnight and then processed. The leukocyte fraction was incubated with our pan-CTC antibody capture cocktail, labeled with biotinylated secondary antibody, followed by enrichment using our streptavidin coated microfluidic channels. Enriched cells were stained for DAPI, cytokeratin, CD45, MLH1/MSH2/MSH6/PMS2 and CEE-Enhanced (pan-CTC stain). After automated fluorescence scanning, spiked tumor cells within the microchannel were identified and average MMR intensities were quantified for each cell.

Results

CTC antibody capture and staining. CTCs are captured in transparent microfluidic channels and can be viewed in situ by fluorescent microscopy. CTCs can be analyzed via immunofluorescence (IF).

Fig. 1: Biocept platform for CTC capture and staining. MMR proteins are detected in blood samples enriched for MMR positive colorectal cancer cells by flow cytometry. MMR status in circulating tumor cells (CTCs) is determined by automated microscopy.

Fig. 2 MMR Proteins Expression in Colorectal Cancer Cell Lines. Flow cytometry was used to determine MSH1, MSH2, MSH6, and PMS2 expression level in HT29 (A), LoVo (B), and HCT15 (C).

Analytical Validation

A.

B.

C.

D.

Fig. 3 MFI Distribution of MMR Positive Colorectal Cancer Cells in Cell Lines. This scatter plot (mean with SD) shows the mean fluorescent intensity (MFI) measurement of MMR proteins, MLH1(A), MSH2(B), MSH6(C), and PMS2(D) protein, in blood spiked colorectal cancer cell lines enriched and quantified in the Biocept microfluidic device.

Fig. 4 Percentage of MMR Positive Cells in Colorectal Cancer Cell Lines. A bar graph shows a representative amount of cells, in blood spiked colorectal cancer cell lines enriched and quantified in the Biocept microfluidic device.

Fig. 5 MMR Stain on Microfluidic Enriched Colorectal Cancer Cells Spiked into Blood and Patient Samples. Images of cancer cells are shown with the antibody stains, cytokeratin (green) and MMR protein (orange). Images were taken through HTI system at 10x.

Conclusions

- The Biocept MMR assay can accurately detect MMR proficient and deficient cells spiked into whole blood samples as well as in patient samples.
- The ability to detect MMR deficient cancer cells in blood affords an opportunity to identify patients likely to benefit from immune therapy, as well as a way to monitor treatment efficacy.

References