AMP
2019
Category: Hematopathology
Poster# H015
Abstract# 16788

Mutant-p53 antibody stains cytokeratin negative CTCs enriched and detected with a “Pan-CTC” antibody cocktail

Steven (Hao-Ching) Hsiao, PhD, David Young, Robbie D. Schultz, PhD, Julie Ann Mayer, PhD, Lyle Arnold, PhD, Jason Poole, PhD and Tony J Pircher, PhD
Biocept Inc, San Diego CA

Biocept
Completing the Answer™

Introduction

Circulating tumor cells (CTCs) are heterogenous but can simply be divided into epithelial-like and stem-like CTCs. Stem-like CTCs comprise tumor cells entering the epithelial to mesenchymal transition (EMTs) and circulating cancer stem cells (CSCs). While CTCs are commonly identified as cytokeratin positive and CD45 negative, we, in contrast, enrich and detect CTCs using an antibody cocktail (pan-CTC marker) targeting cell surface markers expressed on various CTC phenotypes[1]. We investigated the specificity of the pan-CTC antibody cocktail in blood, and at the same time, used anti-mutant-p53 antibodies to demonstrate the origin of identified tumor cells. P53-mutations are found in more than 50% of tumors, and anti-mutant-p53 antibodies recognizes inactive p53 protein caused by mutations in the DNA-binding domain / hotspot region, which consist of >85% of all p53 mutations.

Methods

Flow cytometry was performed on buffy coat with and without tumor cells, to measure pan-CTC antibody cocktail specificity. Detection was accomplished by labeling with PE-labeled anti-mouse-IgG. Blood samples were collected in CEE-Sure blood collection tubesTM (BCT) and shipped at room temperature. Control blood samples were spiked with tumor cells containing p53 hotspot mutations and control cell lines. Buffy coat was prepared by density centrifugation. Samples were labeled with pan-CTC capture antibodies (10 antibodies targeting 10 antigens, including EPCAM), followed by labeling with biotin-anti-mouse IgG. Samples were then loaded into Biocept’s streptavidin coated microfluidic device for CTC enrichment. Staining against CTC markers was performed in the microfluidic channel, including pan-cytokeratin, CD45, mutant-p53 and pan-CTC markers. Microfluidic channels were analyzed by automated imaging system. CTC from patients samples were enumerated using Biocept’s streptavidin coated microfluidic device then stained with pan-cytokeratin, CD45, mutant-p53 and pan-CTC markers with the same procedure as cell line control samples.

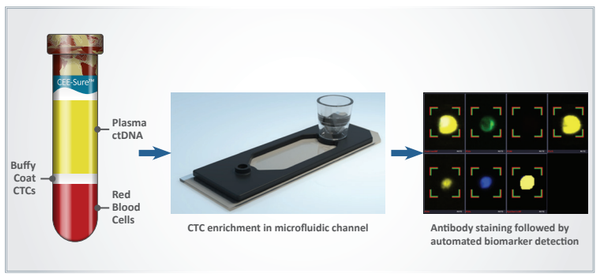

Figure 1. Biocept platform for CTC capture and staining. CTCs are captured in transparent microfluidic channels and can be viewed in-situ by fluorescent microscopy. CTCs can be analyzed via immunofluorescence (IF).

Results

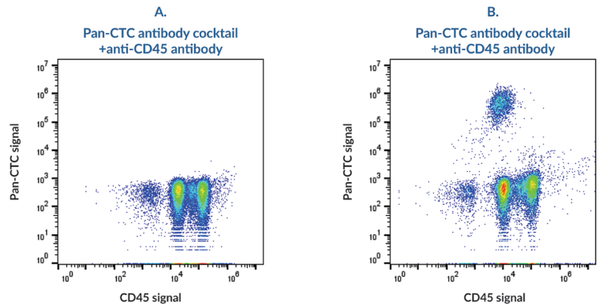

Figure 2. Flow cytometry data demonstrating for the specificity of the pan-CTC antibody cocktail. (A) PBMCs only; (B) PBMCs were spiked with a BT474 breast cancer cell line.

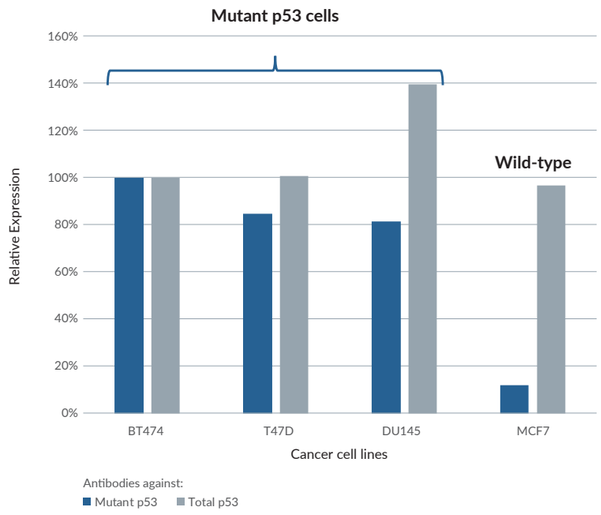

Figure 3. Flow cytometry data shows the expression of p53 (total) in all cell lines and p53 mutant in p53 mutant positive and negative cell lines. BT474, T47D, and DU145 are p53 mutant cell line but MCF7 has wild type p53. p53 and mutant p53 expression was normalized against BT474.

Results

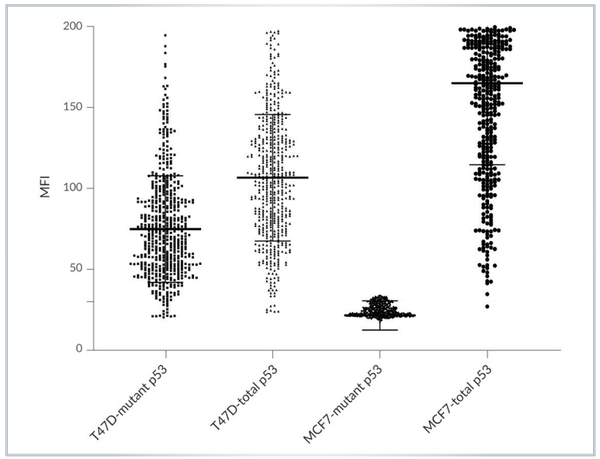

Figure 4. Mutant p53 antibody specifically stain mutant p53 T47D cells in microfluidic channels. Each dot represents MFI signals/individual cell.

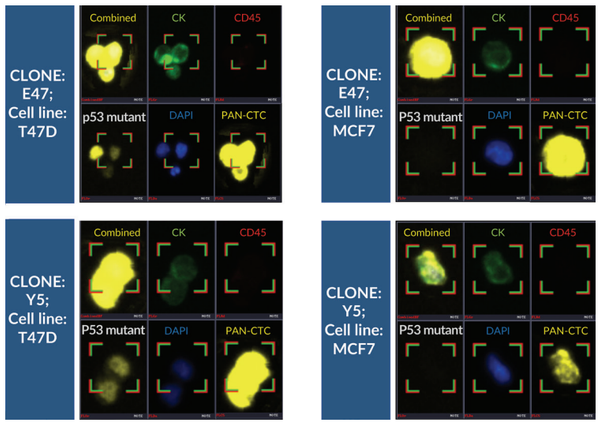

Figure 5. **Examples of mutated p53 staining by anti-p53 mutant antibodies.** T47D was used as the p53 positive control and MCF7 was used the p53 wild type control.

Results

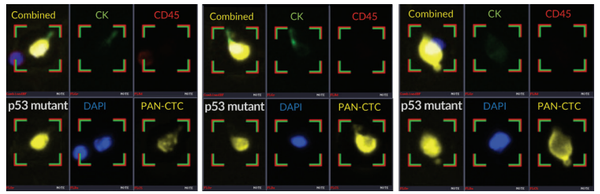

Figure 6. A representation of patient sample with p53 mutant positive CTC. Images of individual cells were obtained by Bioview® imaging system.

Patient	Mutant p53+/CK-	Mutant p53+/CK+	p53 mutation site
Patient #1 (Prostate)	Detected	Not detected	No detection
Patient # 2 (Lung)	Detected	Detected	C277F
Patient # 3 (Pancreatic)	Detected	Detected	R273H
Patient # 4 (Breast)	Detected	Not detected	No detection*
Patient # 5 (Ovarian)	Detected	Not detected	P152T
Patient # 6 (Breast)	Detected	Detected	R248Q

*AKT1 SNP was detected.

Figure 7. Detection of mutant p53 positive cells and NGS confirmed hot-spots mutation. Target Select NGS (Next-Gen Sequencing Breast Panel) powered by Oncomine™ Thermo Fisher Scientific was used to provide sequence information of p53 mutations in patient’s plasma samples that were positive for p53 mutation antibody staining.

Conclusions

- The pan-CTC antibody cocktail is a unique and highly sensitive tool for isolating and detecting CTCs, without being dependent on size or phenotype.
- Mutant p53 antibodies further confirmed the specific pan-CTC antibody cocktail assisted identification of both cytokeratin positive and cytokeratin negative CTCs.
- The variability of p53 positive cancer type proves the utility of anti-p53 mutant antibodies in identifying CTC with cytokeratin negative pan-CTC positive staining.

References

1. Mikolajczyk SD, Millar LS, Tsinberg P, et al. Detection of EpCAM-Negative and Cytokeratin-Negative Circulating Tumor Cells in Peripheral Blood. *J Oncol.* 2011;2011:252361.