Characterization and identification of specific EGFR mutations in circulating tumor cells (CTCs) isolated from non-small cell lung cancer patients using an antibody independent method, ApoStream™

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Abstract

Background: A variety of methods for capture of rare CTCs of unknown origin are available (e.g., simple antibodies to epithelial cell adhesion molecule (EpCAM) and CD45). Using a non-epithelial phenotype definition, a CTC is a nucleated, EpCAM+CD45– cell. However, some CTCs may still capture as they originate from primary tumor cells that have undergone epithelial-mesenchymal transition (EMT). We report here that an antibody independent method, ApoStream™, utilized antibody-free methods to isolate CTCs from blood.

Methods: Blood was obtained from pan-NSCLC patients and processed using ApoStream™. CellSearch™ was also obtained from pan-NSCLC patients and processed using ApoStream™. CTCs identified by ApoStream™ were validated with a multiplexed immunofluorescent assay and laser capture cytometry was applied to identify multiple combinations of markers without negative staining for CK and CD45. To determine specific EGFR mutations, captured CTCs were analyzed using improved and Complete Exon 20 and 21 Amplification (ICE-PCR) sequencing and (ICE) gene panel. Results: Blood samples from 33 NSCLC patients and 3 healthy volunteers were processed. ApoStream™ identified 1 (1%) CTCs and CellSearch™ identified 1 (1%) CTCs. From 7 patients, CTCs were detected in 6 (86%) and 4 (57%) using ICE-PCR and CK+/CD45– cells, respectively. EGFR mutations detected and sample identifiers are shown in Table 1. ApoStream™ results were concordant with CellSearch™ in 20 specimens and discordant in 1 specimen.

Results: The cell type analysis results are shown in Table 2. % EpCAM+ CK+/CD45– cells detected in CTCs isolated by ApoStream™ were compared to CellSearch™. ApoStream™ detected higher % EpCAM+ CK+/CD45– cells than CellSearch™ in all specimens. ApoStream™ detected 1% CK+/CD45– cells in 20 specimens while CellSearch™ detected 1% CK+/CD45– cells in 9 specimens. ApoStream™ detected 1% CK+/CD45– cells in 20 specimens while CellSearch™ detected 1% CK+/CD45– cells in 9 specimens.

Summary & Clinical Significance

A novel, antibody independent platform ApoStream™ successfully isolated CTCs from the blood of patients with advanced NSCLC. In a side-by-side comparison, ApoStream™ isolated non-CX/CD855 NSCLC CTCs compared to the CellSearch™ platform in 3 out of 4 NSCLC patient samples with detectable CK+/CD45– cells, neither system detected CTCs in 1 patient sample.

Phenotypic immunofluorescence analysis of cells isolated by ApoStream™ revealed the presence of CK+/CD45– CTCs as well as CK−/CD45+ CTCs cells. Methods: Of 15% of CTC DNA specimens, 45% of CTCs were detected in NSCLC samples as compared to 0% in healthy donors. Additional sample sizes are being recruited to establish CTC enumeration cut-off.

Percent cells expressing EpCAM varied from 0% to 100% in OK−/CD45− cells, from 0% to 7% in CTCs, and from 0% to 85% in CD45+ cells, thus confirming that ApoStream™ screens cells that could be identified by EpCAM-based technologies.

The use of ICE-COLD-PCR coupled with standard Sanger sequencing allowed detection of EGFR Exon 19 mutations in CTCs isolated by ApoStream™. Sanger sequencing did not increase the sensitivity of detecting EGFR Exon 19 mutations in CTCs from tissue-positive patients compared to standard ICE-COLD-PCR. ICE-COLD-PCR did not detect any cells with EGFR mutations.

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Representative Immunofluorescent Images of Cells Isolated by ApoStream™

Table 1: Summary of CTC Enumeration and Mutation Analysis Results

Table 2: CTC Cell Type Analysis

Table 3: CTC mutational analysis

Figure 1: ICE-COLD-PCR workflow

Figure 2: ApoStream™ Technology

Figure 3: ICE COLD-PCR

Figure 4: Representative Immunofluorescent Images of Cells Isolated by ApoStream™