Adenocarcinoma accounts for 40% of all lung cancers. It is also the most common subtype of non-small cell lung cancer (NSCLC)\(^1\).

CellSearch\textsuperscript{™} CTC system using EpCAM and CK biomarkers has been FDA approved only for metastatic prostate, breast and colorectal cancers\(^2\).

Clinical biomarkers best suited for lung cancer CTC capture and detection are not clearly known. Active research is ongoing to fill this void.

Current CTC testing is intended for patient’s peripheral blood. CTC detection in pleural effusion is a new and emerging application\(^3\).

Cell-surface vimentin (CSV) is an universal cancer biomarker of epithelial mesenchymal epithelial (EMT) CTCs. CSV application in lung cancer CTCs has not been previously reported\(^4\).

We applied CytoQuest\textsuperscript{™} CR positive enrichment system to assess CSV and PanCK monoclonal antibodies for capture and detection of lung adenocarcinoma EMT CTCs.

Pleural effusion of lung adenocarcinoma patient was collected and prepared by density gradient centrifugation using Leucosep\textsuperscript{®} (163290P, Greiner Bio-One) and Histopaque\textsuperscript{®}-1077 (10771, Sigma-Aldrich).

Nucleated cell fraction was harvested and re-suspended in Wash Medium.

Re-suspended nucleated cells were loaded into the CytoQuest\textsuperscript{™} CR System and EMT CTCs were captured by biotinylated CSV monoclonal antibody immobilized on CytoChipNano (U0095, Abnova).

Immunofluorescence staining for detecting EMT CTCs was performed using PanCK, CD45 (KA4439, Abnova) and DAPI as the instruction protocol.

Imaging was performed using Nikon Eclipse Ti-E fluorescent inverted microscope.

Figure 1. Representative images of CSV Transformed EMT CTCs (white arrow) and WBCs (yellow arrow) from pleural effusion of lung adenocarcinoma patient. CSV transformed EMT CTCs were detected by using immunofluorescence staining for PanCK (FITC, green), CD45 (PE, orange) and Nucleus (DAPI, blue).

EMT CTC Counts: In 20 mL pleural effusion of lung adenocarcinoma patient, 62 cells counted as CSV Transformed EMT CTCs (CSV\textsuperscript{+} capture, and PanCK\textsuperscript{+}, CD45\textsuperscript{-}, DAPI\textsuperscript{+} detection).

Liquid biopsy using circulating tumor cells (CTCs) is a non-invasive method for detecting and monitoring cancer progression and treatment response.

Tumor metastasis, drug resistance and disease relapse are highly correlated with epithelial-mesenchymal transition (EMT).

Biotinylated CSV capture and PanCK-FITC detection antibodies are highly effective in isolating pleural effusion EMT CTCs of lung adenocarcinoma.

This is the first case report of novel CSV expression in lung cancer pleural effusion EMT CTCs, consistent with previous CSV findings are in breast, colorectal and liver cancers\(^5\text{-}7\).

Future study should include simultaneous isolation of lung adenocarcinoma patient’s peripheral blood and pleural effusion EMT CTCs using CSV and PanCK antibody combination.
References


