Detection of Lung Cancer From Plasma Using the Biosignature of Circulating Microvesicles

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Abstract

Circulating microvesicles (cMV) are cell-derived structures that are abundantly present in the blood. Tumor cells produce large quantities of cMV, and their amount has been shown to correlate with tumor invasiveness and resistance to therapy. This study attempts to understand the origin, composition, and potential clinical utility of cMV, analyzing their protein composition in patients with non–small cell lung cancer (NSCLC) and identifying a cMV-based biosignature that can be used to predict the presence of tumors from a blood sample.

cMV were isolated from plasma samples that were obtained from an initial cohort of 65 patients with NSCLC and from 46 control samples. A discovery panel of 63 specific biomarkers was used to develop an assay with high specificity and sensitivity based on cMV surface proteins. Using a novel cMV-based multiplexed analysis platform we optimized a threshold level for 4 significant cMV subpopulations to effectively distinguish lung cancer patients from controls.

The multiplex assay included 1 general cMV marker (CD81) and 3 lung cancer–associated biomarkers: the lung epithelial C-type lectin members SPD and SPA, plus the sialoprotein osteopontin. The decision tree analysis, based on specific cut offs, showed a sensitivity of 81% and a specificity of 90%.

These results provide initial evidence that the identification of biosignatures in distinct subpopulations of cMV may offer a powerful blood-based approach for the detection and monitoring of specific disease states, such as non small cell lung cancer.

Methods

Figure 1. For SEM images H1975 cells were seeded at 20% confluency on glass plates coated with poly-L-lysine. Cells were washed twice with medium without additives, and initially fixed in 2.5% glutaraldehyde. A secondary osmium tetroxide fixation was performed followed by a series of ethanol dehydration steps. Specimens were mounted on the SEM support with silver paste. Images were taken using an FEI XLF30-FEG scanning electron microscope equipped with an Everhart-Thornley secondary-electron (SE) detector; backscattered electron (BE) detector; EDAX Si(Li) EDX detector with ultra-thin window for light element analysis

Figure 2. confocal images of microvesicles (MV) purified from VCaP cells were, labeled with BodiPY and captured with magnetic beads coated with CD9/CD63/CD81 antibody.

Figure 3. Detection of lung cancer using biomarkers were performed on plasma from 65 NSCLC patients and 46 age-matched contrrols using 63 previously selected markers.

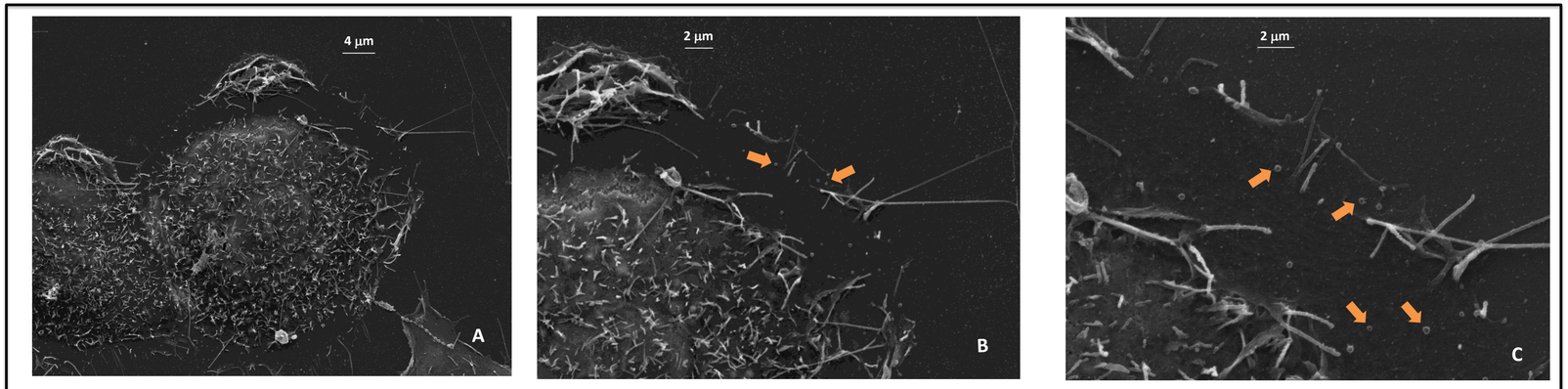

FIGURE 1. SEM images of H1975 lung cancer cells. Arrows shows microvesicles produced by the cells. (A) x1500 magnification (MG), (B) x2000 MG, (C) x3000 MG.

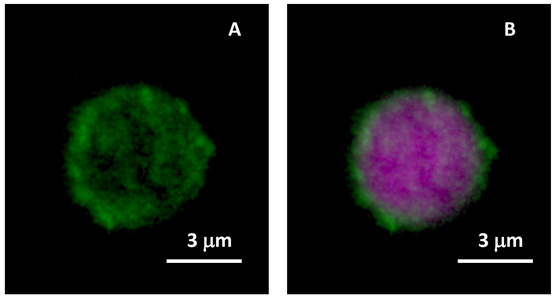

FIGURE 2. Confocal Image of immobilized MV. (A) shown in green MV only. (B) merged image with the magnetic bead colored in red.

	All data
True positive	56
True negative	38
False positive	4
False negative	13
Total samples	111
Sensitivity	81%
Specificity	90%
Accuracy	85%

FIGURE 3. Summarizing table. Using 1 MV specific marker (CD81) and 3 lung-cancer associated biomarkers (C-type lectin members SPD, SPA, plus the sialoprotein osteopontin) low grade NSCLC patients were identified , with 81% sensitivity, 90% specificity and 89% accuracy

Results

- Cancer cell are able to produce homogeneous shaped microvesicles sized between 200 to 500 nm.
- These microvesicles can be immobilized on magnetic beads using specific markers.
- Using this capturing technique, we have identified an initial biomarker signature for non small cell lung cancer patients. This signature discriminated lung cancer patients from healthy controls with 81% sensitivity, 90% specificity and accruacy of 85%

Conclusions

These results provide initial evidence that the identification of biosignatures in distinct subpopulations of cMV may offer a powerful blood-based approach for the detection and monitoring of specific disease states, such as NSCLC.

In this report, we have identified 1 MV specific marker (CD81) and 3 lung-cancer associated biomarkers (C-type lectin members SPD, SPA, plus the sialoprotein osteopontin) that together, can differentiate 65 patients with low grade NSCLC from 46 control patients.