

Novel Non-Invasive Detection Test Identifies Early Stage Non Small Cell Lung Cancer (NSCLC): Utilizing 3D Analysis of Cellular Global DNA Methylation in Sputum

Objective: Non-invasive, non-radiographic screening for Non Small Cell Lung Cancer (NSCLC) is sparse and sputum cytology is not reliable for screening purposes. However, sputum is a valuable source of exfoliated respiratory epithelial cells that are transformed early in lung carcinogenesis. Characterized by global DNA methylation imbalances, with malignant cells being hypomethylated and containing 20–60% less genomic 5-methylcytosine (5mC) (an epigenetic biomarker) than non-malignant cells. By developing a novel test that detects and quantifies aberrantly hypomethylated cells in induced sputum, we investigate the efficacy of this test as a screening tool for detection of early-stage NSCLC.

Methods:

The test has 3 steps: 1) Isolation of cells from sputum and fluorescence labeling on microscopic slides 2) confocal imaging 3) computational image/data analysis of labeled specimens. Cells undergo immunofluorescence labeling with antibody specific to 5mC and then they are counterstained with 4',6-diamidino-2-phenylindole (DAPI) to delineate global nuclear DNA (gDNA). Confocal scanning is performed by imaging optical sections through the cells at 500nm increments.

Imaged cells are processed for 3D segmentation of cell nuclei. Thereafter, fluorescence 5mC and DAPI signals within the cell nuclei are measured, which generates two parameters for each imaged cell (a) global 5mC intensity and (b) gDNA signals (represented by DAPI). 3D image analysis is performed using a dedicated algorithm for multi-parametric high-content analysis. A co-distribution map (2D scatter plots) of 5mC + gDNA signals is generated. The angle under the regression line is automatically calculated as the second parameter. The proportion of aberrantly hypomethylated cells versus normal epithelial cells in a sputum sample determines the likelihood of NSCLC existence.

Results: 88 patients were enrolled. 12 (14%) healthy patients, 34 (39%) high-risk patients with benign chronic lung disorders (10 COPD, 24 IPF), and 43 (49%) NSCLC patients (24 stage I-II, 19 stage III-IV). The angle under the regression line associated with a cancer diagnosis was < 6.41 and the optimal threshold value for the percentage of abnormal cells associated with a cancer diagnosis was 30.30% for 5mC and 38.46% for 5mC + DAPI. The test identified early-stage NSCLC and distinguished it from the high-risk patient group with 96% (95% CI [76.4, 99.9]) sensitivity and 41% (95% CI [22.8, 61.5]) specificity.

Conclusions:

We have developed a novel, highly sensitive, screening test for NSCLC, utilizing sputum for the detection of malignancy. Our non-invasive test has a 96% sensitivity in diagnosing lung cancer by combining immunofluorescence, confocal imaging, and high-content 3D image analysis. This method, allows for the determination of the quantity of hypomethylated cells, based on the load and distribution of global 5mC (5mC + gDNA represented by DAPI) in the nuclei of epithelial cells isolated from sputum. Given the ease with which sputum samples can be obtained from patients, as well as no exposure to radiation, our novel test may impact lung cancer screening, evaluation of pulmonary nodules, evaluating disease recurrence in post-resection patients and cancer surveillance algorithms.

Healthy vs High-Risk vs Lung Cancer

(% cells above and below parameter cut-off)

