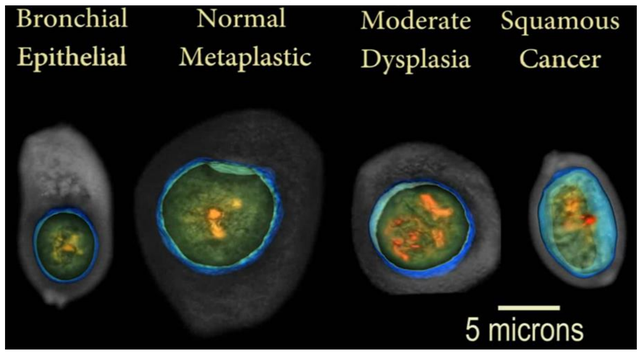
3D Morphometric Detection of Mismatch Repair Deficiency in Human Lung Adenocarcinoma Cell Lines using the Cell-CT® Platform

Abstract #:14118 D. J. Sussman, L. Kelbauskas, M. Meyer, R. Katdare, A. Nelson

Background

Cell-CT Processing: Automatically analyzes cells in true 3D with isometric, sub-micron resolution



Motivation: Mismatch repair protein deficiency (MMR-D) is proving to be a predictive biomarker for the efficacy of immune checkpoint inhibitor therapy. While multiple molecular tests are available for the detection of MMR-D, such as staining for expression levels of mismatch repair pathway proteins (MLH1, MSH2, MSH6, and PMS2), they require invasive biopsy and are often not applicable for early stage disease.

Studies have demonstrated that MMR-D results in histological and morphological changes. The Cell-CT® platform produces isometric, high-resolution 3D images of cells in liquid biopsies, such as sputum, where published studies demonstrated 92% sensitivity and 95% specificity to biopsy-confirmed lung cancer across different stages. This study reports the development of morphology phenotype based classifiers for lung cancer cell lines that have been engineered to exhibit MMR-D.

Methods

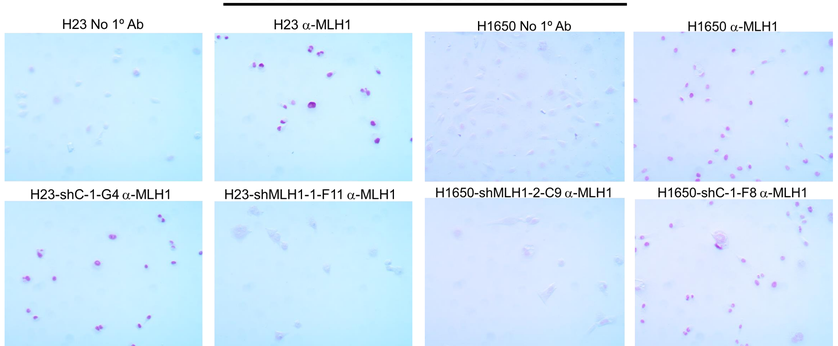
Study Materials:

Two human lung cancer adenocarcinoma cell lines, NCI-H23 and NCI-H1650, were transduced with lentiviral particles expressing either scrambled or MLH1 shRNAs and selected for puromycin resistance. Individual clones were isolated and screened for MLH1 expression. Several scrambled shRNA clones, with parental levels of MLH1, and several shMLH1 clones, with a range of 86 to 97% suppression of MLH1, were expanded, fixed, and analyzed using the Cell-CT® platform. Over 1,000 cells from several harvests of each cell line were used to measure 845 different 3D structural features for each cell.

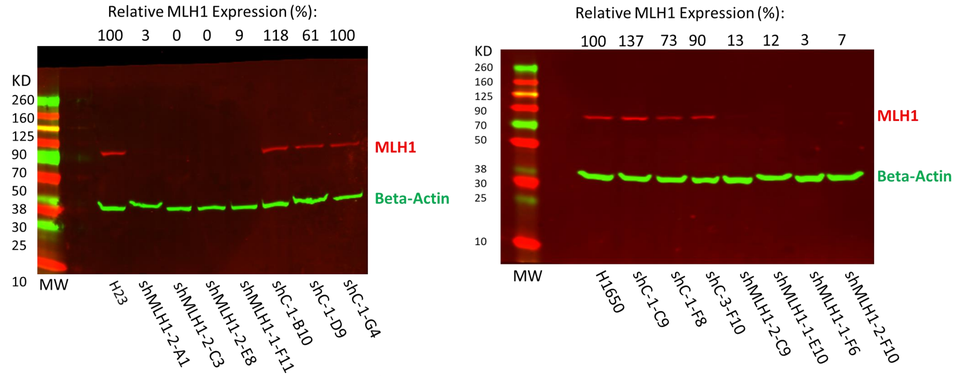
Classifier Development:

- 845 morphometric features for each 3D cell image
- Ground truth defined by the cell line
- Adaptive boosting method for classifier creation

Results and Conclusions



IHC Staining of parental cell lines and representative scrambled shRNA and shMLH1 clones with an anti-MLH1 antibody



Western blot quantitation of MLH1 expression in parental cell lines and scrambled shRNA and shMLH1 clones.

The performance of the classifier to test the degree to which the features discriminate between control scrambled shRNA and shMLH1 clones was characterized by the area under the ROC curve (aROC):

H23 Clone:	shMLH1-2-C3	shMLh1-2-A1	shMLH1-1-F11	shMLH1-2-E8
aROC:	0.86	0.81	0.81	0.84

H1650 Clone:	shMLH1-2-C9	shMLh1-1-F6	shMLH1-2-F10
aROC:	0.83	0.8	0.9

3D Cell morphology shows promise as a means of identifying MMR-D in malignant cells.

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