High-dimensional morphology analysis reveals new insights in melanoma cell heterogeneity and enables label-free phenotyping

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KEY POINTS
- The REM-I platform characterizes & sorts cells based on high-dimensional morphology analysis without labels, eliminating the need for specific biomarkers.
- We demonstrate that high-dimensional morphology can distinguish mesenchymal vs melanocytic cells in both patient-derived cell lines and dissociated tumor biopsies.
- An AI-based random forest classifier can predict cell phenotype based on morphology alone, verified by single-cell transcriptional information.

INTRODUCTION
- Melanomas are the deadliest skin cancers, in part due to cellular plasticity and heterogeneity within the tumors.
- The Deepcell platform enables high-dimensional morphology analysis and enrichment of unlabeled single cells using artificial intelligence (AI), advanced imaging, and microfluidics, enabling high-resolution profiling of population heterogeneity.
- We imaged and analyzed 18 patient-derived melanoma cell lines representing both mesenchymal and melanocytic phenotypic states.
- High-dimensional morphological analysis showed distinct clusters for each phenotype, indicating distinct morphotype for each phenotype.
- We developed a random forest classifier to identify the top differential morphological features between the different cell lines, thereby providing a label-free means of phenotyping melanoma samples.
- The morphological analysis of the cell lines uncovered significant variability in pigmentation; a random forest classifier distinguished pigmented vs non-pigmented cells with >90% accuracy.
- Application of the classifier to images of dissociated tumor biopsies identified their phenotype, which was verified by scRNA-Seq, demonstrating the application of a label-free phenotyping using morphology alone in clinical samples.

METHODS

RESULTS

A random forest classifier can predict phenotype based on morphology alone

A. Transcriptional label vs Classifier Prediction

B. Transcriptional label vs Classifier Prediction

C. Mesenchymal vs Melanocytic

D. Mesenchymal vs Melanocytic

E. Mesenchymal vs Melanocytic

F. Mesenchymal vs Melanocytic

G. Mesenchymal vs Melanocytic

H. Mesenchymal vs Melanocytic

I. Mesenchymal vs Melanocytic

J. Mesenchymal vs Melanocytic

K. Mesenchymal vs Melanocytic

L. Mesenchymal vs Melanocytic

M. Mesenchymal vs Melanocytic

N. Mesenchymal vs Melanocytic

O. Mesenchymal vs Melanocytic

P. Mesenchymal vs Melanocytic

Q. Mesenchymal vs Melanocytic

R. Mesenchymal vs Melanocytic

S. Mesenchymal vs Melanocytic

T. Mesenchymal vs Melanocytic

U. Mesenchymal vs Melanocytic

V. Mesenchymal vs Melanocytic

W. Mesenchymal vs Melanocytic

X. Mesenchymal vs Melanocytic

Y. Mesenchymal vs Melanocytic

Z. Mesenchymal vs Melanocytic

Figure 2. High-dimensional morphology distinguishes mesenchymal vs melanocytic cell lines. (A) 18 patient-derived cell lines from metastatic melanomas representing mesenchymal and melanocytic phenotypes were imaged and analyzed on the Deepcell platform. The phenotype of each cell line was based on mesenchymal or melanocytic gene expression signatures from bulk RNA-Seq data. (B) Images of cells indicate qualitative morphology differences detectable by eye, with melanocytic cells appearing smaller and mesenchymal cells are more granular. (C) Leiden clustering highlights the morphological diversity across the patient derived cell lines. (D) High-dimensional morphology UMAP showing distinct clusters for mesenchymal (orange) and melanocytic (green) cells, further illustrated by density plots. Inspection of the individual cell lines shows multiple morphotypes within each phenotype.

Figure 3. A random forest classifier can predict phenotype of melanoma cells with up to 77% accuracy. (A) We developed a random forest classifier to predict the phenotype of melanoma cells based on the images taken on the Deepcell platform. 8 cell lines with the most clear transcriptional score were used to train the classifier, which was then tested on an additional 12 cell lines. The classifier can predict the phenotype with up to 77% accuracy (B) The classifier provides single-cell phenotype information, noted in the percent of cells classified as each phenotype for each cell line. (C) Local binary pattern (LBP) features, a metric of texture and granularity, are the top differential morphometric features distinguishing mesenchymal and melanocytic phenotypes. (D) The distribution of the top feature is shown, suggesting mesenchymal cells (blue) have higher measures of granularity compared to melanocytic cells (orange). DJ, deep learning.

CONCLUSIONS
- High-dimensional morphology analysis shows multiple morphotypes for melanocytic and mesenchymal cells.
- These morphological differences were used to develop a Melanoma Phenotype Classifier that can predict phenotype with up to 77% accuracy.
- The morphometric features also identified a unique pigmented population with up to 95% accuracy.
- The Melanoma Phenotype Classifier can be used to predict the phenotype of dissociated melanoma biopsies.
- These results suggest that high-dimensional morphology can be used to characterize phenotype in a label-free manner, and provide new insights into tumor biology.