Immunofluorescence Image Feature Analysis and Phenotype Scoring Pipeline for Distinguishing Epithelial–Mesenchymal Transition

Shreyas U. Hirway, Nadiah T. Hassan, Michael Sofroniou, Dr. Christopher A. Lemmon, Dr. Seth H. Weinberg*

333 W 10th Ave, Columbus, OH 43210
The Ohio State University
Columbus, OH, USA
hirway.2@buckeyemail.osu.edu

Epithelial-Mesenchymal Transition (EMT) is a key biological process in which epithelial cells transdifferentiate into mesenchymal cells that is involved in normal processes such as embryonic development, tissue regeneration, and wound healing, as well as pathological processes, such as cancer metastasis.[1,2] We developed an image processing pipeline to analyze images of cells undergoing EMT and characterize transitions along the EMT progression using various biomarkers. [3] Mammary epithelial cells were incubated with 0, 2, 4, or 10 ng/mL Transforming Growth Factor β1 (TGF-β1) and cultured for 72 hours. Cells were fixed, stained, and imaged for E-cadherin, actin, fibronectin, and nuclei via immunofluorescence microscopy. qRT-PCR was also performed by extracting RNA from cells after 72 hours of treatment and processing for relevant biomarkers. Feature selection was performed on the 80 image set of composites and individual biomarkers using the MATLAB Speeded Up Robust Feature (SURF) extractor.[4] Using a machine learning approach, a training group of epithelial (0ng/mL) and mesenchymal (10ng/mL), and a testing group of 2ng/mL and 4ng/mL was created to define relevant image features. The feature space average of the epithelial and mesenchymal training groups were calculated, and Euclidean distance analysis was performed on the image features to measure the "closeness" of each image to the two training groups. We find that the control treated images were distinct from the 10 ng/mL-treated, while the moderately dosed testing images possessed characteristics similar to both treatment groups in the training group, illustrating a gradual transition path from epithelial to mesenchymal cells and consistent with multiple hybrid EMT states. qRT-PCR measurements of EMT-associated biomarkers correlated with the image analysis. Overall, we identified image pipeline characteristics for feature extraction and quantification of immunofluorescence images to distinguish cell monolayers in multiple stages of the EMT program.

References

1. Katsuno Y, Lamouille S & Derynck R (**2013**). TGF-β signaling and epithelial–mesenchymal transition in cancer progression. Curr Opin Oncol 25, 76–84
2. Hao Y, Baker D & ten Dijke P (**2019**). TGF-β-mediated epithelialmesenchymal transition and cancer metastasis. Int J Mol Sci 20, 2767.
3. Hirway, S. U., Hassan, N. T., Sofroniou, M., Lemmon, C. A., & Weinberg, S. H. (**2021**). Immunofluorescence Image Feature Analysis and Phenotype Scoring Pipeline for Distinguishing Epithelial–Mesenchymal Transition. Microscopy and Microanalysis, 1-11.
4. Bay H, Ess A, Tuytelaars T & Van Gool L (**2008**). Speeded-up robust features (surf). Comput Vis Image Underst 110, 346–359.