Measuring phenotypes of Barrett’s esophageal cancer cells at the single cell level

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Introduction

Barrett’s esophagus is a condition in which the tissue lining the esophagus is replaced by tissue that is similar to the lining the intestine. In many cases, esophageal adenocarcinoma is not identified until later stages when treatments are not always effective. Although the causes of Barrett's esophagus are not known, a condition known as GERD, acid reflux, is a risk factor. Estimates show that approximately 10 to 20% of Americans experience GERD on a weekly basis. Furthermore, due to its well characterized normal-to-cancer progression path, the Barrett's esophagus can be used as a model system for cancer induction and development studies. By studying the proliferation and other phenotypes of Barrett’s esophageal cells from different precancerous and cancerous developmental stages at the single cell level, our objective at the Center for Biosignatures Discovery Automation is to identify and characterize early aberrant transcriptional and carcinogenic.

Motivation

- Current cellular research is predominantly based on bulk analyses.
- The results are expressed as population averages thereby masking the significance of intrinsic cellular heterogeneity.
- Many diseases, including cancer, originate in few aberrant progenitor cells.

Our Approach

- Measuring the concentrations of various metabolites by means of extracellular optical sensors in a hermetically sealed microchamber containing the cell.
- We produce the hermetic seal by placing a lipped lid containing the sensor on top of the microwell with the cell.

Methods

Step 1: Cell Loading
a) A cell is aspirated into the micropipette tip.
b) The tip is lifted and the reservoir with the microwell substrate at the bottom is moved to align with the objective.
c) The cell is dispensed from the micropipette tip into a microwell.

Step 2: Drawdown Experimental Setup
a) The hermetically sealed microchamber containing the cell is produced by placing the lid on top of the well and exerting a force of about 40-70 Newtons on the lid through the piston.
b) A compliant layer is placed between the lid and the piston to ensure equal force distribution across the lid.

Step 3: Harvesting and PCR Analysis
1. Sorting KAOS to plate cell-cycle-sorted single-cell level.
2. Cell collection PCR cap by single cells using and grow into cap.
3. RNA isolation.
4. Reverse Transcription.
5. qPCR.
7. Data analysis.

Results

The results are expressed as population averages thereby showing the importance of the single cell approach.

Conclusions and Future Directions

- We have successfully measured oxygen consumption rates of human esophageal epithelial cell lines, CP-A is a metaplastic and CP-C is a dysplastic cell line.

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