**Introduction**

Lung cancer is the leading cause of cancer-related death. It is the second most common cancer in both men and women. Non-small cell lung cancer (NSCLC) is the most common lung cancer at ~85%; with 40% adenocarcinomas and of these 25% are caused by a KRas mutation.

**Background**

Lung cancer metastatizes by way of epithelial to mesenchymal transition (EMT). Lung cancer can exhibit a range of epithelial and mesenchymal characteristics. Epithelial cells have polarity and tight cell junctions. Mesenchymal cells lack polarity and are more invasive. The lab has previously derived a panel of cell lines from the genetically engineered mouse KrasG12D/+; p53R172H/R172H (KPMUT): 393P and 344SQ. These cell lines were thoroughly characterized and it was found that 393P is relatively epithelial and 344SQ is relatively mesenchymal (Don Gibbons, 2009).

More recently, the lab has derived a new panel of cell lines from KrasG12D/+; p53R172H/R172H (KPMUT) mice: 7064P, 7362P, and 7578P. This project aims to characterize the novel cell lines on a scale of epithelial to mesenchymal using the known 393P and 344SQ cell lines as standards.

**Results**

<table>
<thead>
<tr>
<th>NSCLC</th>
<th>Adenocarcinoma</th>
<th>Kras</th>
<th>EGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous Cell</td>
<td>25%</td>
<td>40%</td>
<td>-</td>
</tr>
<tr>
<td>Large Cell</td>
<td>10%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>40%</td>
<td>-</td>
<td>22%</td>
</tr>
<tr>
<td>Unknown</td>
<td>36.4%</td>
<td>25%</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 1:** A model of epithelial to mesenchymal transition which shows loss of epithelial marker and gain of mesenchymal markers.

**Figure 2:** Brightfield images taken with a microscope showing the morphology at 10x and 20x.

**Figure 3:** qPCR of known epithelial and mesenchymal markers. This qPCR was done by extracting RNA from cell lines grown in 2D culture. Reverse transcription was performed to generate cDNA using qScript from QuantaBio. Assays were performed in triplicate using L32 as an internal standard and 393P as the control. Data was normalized and relative expression plotted. Significance was determined by Student's t-test in PrismGraphPad.

**Figure 4:** Western blot of epithelial and mesenchymal markers. Proteins were extracted from 2D culture. Then 30 μg of proteins were added into each well then ran on an 8% acrylamide gel, and transferred to a nitrocellulose membrane. Blocking was performed with 5% milk and primary antibody staining was done overnight. Secondary staining followed the next day and blots were developed and images were taken on the BioRad Chemidoc using chemiluminescence.

**Figure 5:** Migration assay of the five cell lines. Cells were seeded in triplicate into transwell chambers with serum-free media, and the chambers were placed in wells with complete FBS media. The cells were allowed to migrate for 16 hours. Afterwhich, the cells were fixed and stained in crystal violet for 24 hours. Five fields were captured per chamber and cells quantified using ImageJ.

**Conclusions**

Morphologically, 393P and 7578P form tight clusters, while 344SQ, 7064P, and 7362P do not. Moreover, 344SQ, 7064P, and 7362P exhibit protrusions indicative of a mesenchymal nature. At the transcription level the data was inconclusive for the markers looked at; the cell lines showed both over and under expression for epithelial and mesenchymal markers. Functionally, we see different results at the protein level, but cells still seem to have contradictory pathways. Looking at the migration images, all of the cell lines have the capability to mount a migration. The data suggests that the novel cell lines are more mesenchymal than 393P and more epithelial than 344SQ. The novel cell lines still lay within the two extremes of the control.

**Future Directions**

-- Due the variance of data, repeating each of the assays with the same markers will give more confidence of the results.
-- Also, doing the same assays with different markers would lead to more conclusive evidence. These cells may lack plasticity and may not depend on EMT
-- Look at the miR-200 family members by qPCR since they are known to regulate Zeb1
-- Test in vivo as a syngeneic tumor implantation

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