Effect of Long Non-coding RNA on Colon Cancer Migration and Phenotype

Ajay Patel, B.S.\textsuperscript{1,2}, Mason Paas, B.S.\textsuperscript{1}, Stephen O’Brien, MB BCh BAO\textsuperscript{1}, Susan Galanduk, M.D.\textsuperscript{1}

\textsuperscript{1}Price Institute of Surgical Research, The Hiram C. Polk Jr. MD Department of Surgery
\textsuperscript{2}University of Louisville School of Medicine

### Introduction

- 1 in 20 people will be diagnosed with colorectal cancer in their lifetime.
- Colon cancer is the 3\textsuperscript{rd} most diagnosed and 2\textsuperscript{nd} most common cause of cancer-related deaths in the United States.
- Epithelial-to-mesenchymal transition (EMT) is a process by which epithelial cells gain a more mesenchymal phenotype and become more invasive.
- Long non-coding RNAs (lncRNA) exceed 200 nucleotides in length and are not known to code for proteins.
- ZFAS1, a lncRNA, has been shown to play a role in the progression and metastasis of various cancers.
- **Hypothesis**: Knockdown of ZFAS1 would lead to decreased cellular migration for colon adenocarcinoma cell lines HT29, SW480, and Caco2.

### Methods

- Colon cancer cell lines (HT29, SW480, and Caco2) were obtained and grown in appropriate media.
- Cells were transfected with ZFAS1 silencing RNA (siZFAS1) or the negative control (Non-target).
- Transfected cells were harvested and plated for individual assays.
- For scratch migration assay analysis, cells were plated in a 6-well plate at 1 \times 10^5 cells per well and allowed to adhere for 24 hours. At 24 hours, a vertical and horizontal scratch were made and a photo was taken for baseline and at 24-hour intervals thereafter.
- For transwell migration assay, transfected cells were obtained in serum free media. Cells were seeded at 3 \times 10^5 cells/well into 8 μm pore polycarbonate membrane inserts. Seven hundred μL of 10% FBS-media was used in the bottom chamber as a chemoattractant and cells were incubated for 24 hours. After 24 hours, cells were stained and analysis was performed.
- The Mann Whitney Statistic was used to analyze the data.

### Results

#### Scratch Migration Assay

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Time</th>
<th>Percentage migration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29 (Duke’s C)</td>
<td>0 hr</td>
<td>50 ± 5</td>
</tr>
<tr>
<td></td>
<td>120 hr</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>siZFAS1</td>
<td>Non Target</td>
<td>50 ± 5</td>
</tr>
</tbody>
</table>

#### Transwell Migration Assay

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Transwell Migration Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29 (Duke’s C)</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>SW480 (Duke’s B)</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Caco2 (Duke’s Unknown)</td>
<td>30 ± 4</td>
</tr>
</tbody>
</table>

### Results (continued)

#### Conclusion

- Successful transfection was confirmed using quantitative real-time polymerase chain reaction (qRT-PCR).
- For the scratch migration assay, ZFAS1-knockdown samples displayed slower scratch closure compared to the control samples in all three cell lines.
- For the transwell migration assay, ZFAS1-knockdown samples displayed slower migration through the membrane compared to the control samples in all three cell lines.

### Conclusions

- ZFAS1-knockdown leads to decreased cellular migration in colon cancer cell lines: HT29, SW480, and Caco2.
- Therefore, this further indicates that ZFAS1 may play an important role in the process of EMT.
- ZFAS1 should be further investigated as a potential therapeutic target in the treatment of colon cancer.

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\textsuperscript{2}University of Louisville School of Medicine