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

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Introduction

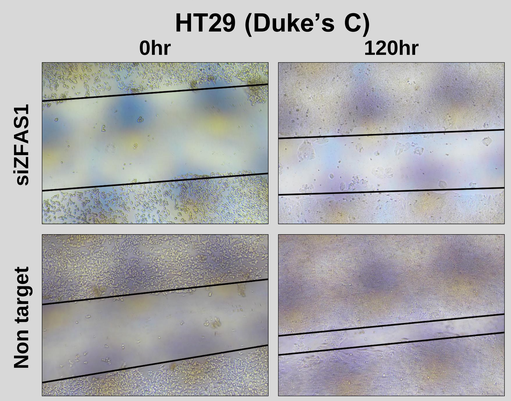
- 1 in 20 people will be diagnosed with colorectal cancer in their lifetime.
- Colon cancer is the 3rd most diagnosed and 2nd most common cause of cancer-related deaths in the United States.
- Epithelial-to-mesenchymal transition (EMT) is a process by which epithelial cells gain a more mesenchymal phenotype 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.

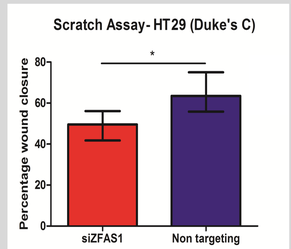
Methods

- 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 x 10^6 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 x 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.

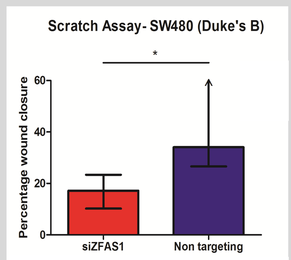
Results

Scratch Migration Assay

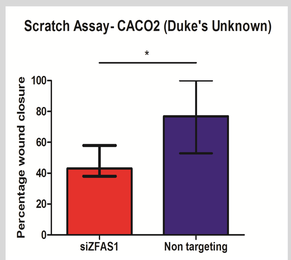




N=9
Median ± Interquartile Range
P=0.06



N=8
Median ± Interquartile Range
P<0.001



N=6
Median ± Interquartile Range
P=0.001

Acknowledgements

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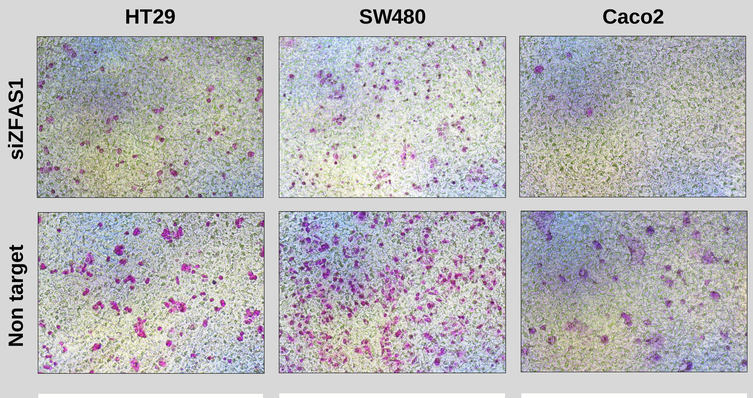
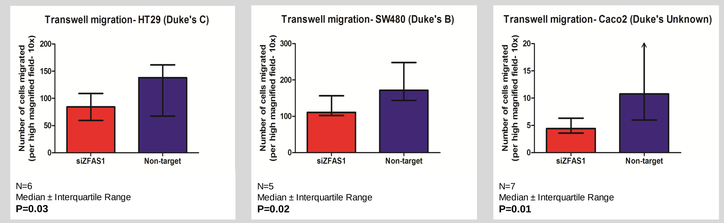
Results (continued)

Transwell Migration Assay





N=6
Median ± Interquartile Range
P=0.03

N=5
Median ± Interquartile Range
P=0.02

N=7
Median ± Interquartile Range
P=0.01

- 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 a important role in the process of EMT.
- ZFAS1 should be further investigated as a potential therapeutic target in the treatment of colon cancer.