ZEB mRNA Expression is Affected by Long Non-coding RNA ZFAS1

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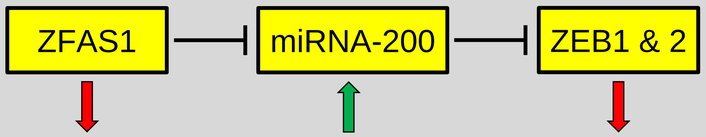
Introduction

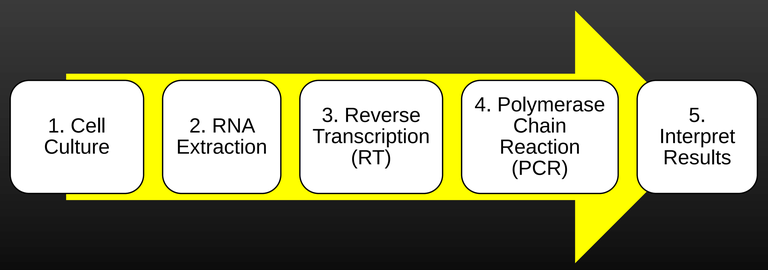
- According to the American Cancer Society, in 2017 approximately 135,000 people were diagnosed with colorectal cancer.
- Survival rates decrease after tumor progression and metastasis. Epithelial-to-mesenchymal transition (EMT) is a process by which metastasis occurs.
- Long non-coding RNAs (lncRNA) have been implicated to play a large role in EMT.
- lncRNAs act as microRNA (miRNA) sponges by interacting with and decreasing their availability.
- miRNAs affect gene expression post-transcriptionally by downregulating mRNA expression.
- lncRNA ZFAS1 has been shown to be upregulated in colon cancer compared to normal adjacent epithelial tissue. Interaction between ZFAS1 and the miRNA-200 family has been shown.
- The miRNA-200 family and ZEB transcription factors are well defined in the literature for playing a major role in EMT by promoting a mesenchymal phenotype, which worsens prognosis.

Hypothesis

We hypothesize that downregulation of ZFAS1 or upregulation of the miRNA-200 family will lead to decreased expression of ZEB1 and ZEB2 mRNA in colon cancer cells lines.



Methods



Methods

- Colon cancer cell lines HT29 and SW480 were acquired (ATCC®, Manassas, VA).
- Cells were plated into 6-well plates at a concentration of 250,000 cells/well and were allowed to adhere for 24 hours.
- Cells were transfected at 24 hours with either ZFAS1 siRNA (A or C isoform), miRNA-200b, miRNA-200c mimics, or negative control siRNA (Dharmacon, Lafayette, CO).
- Both cell lines were harvested for RNA analysis at 24, 48, and 72 hours.
- Total RNA was extracted with miRNeasy Mini Kit (Qiagen®, Germany).
- Reverse transcription was performed using SuperScript™ VILO™ Master Mix (Invitrogen™, Carlsbad, CA).
- PCR was performed using specific TaqMan Gene expression assays (Life Technologies, Carlsbad, CA).

Acknowledgments

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Results

Figure 1)

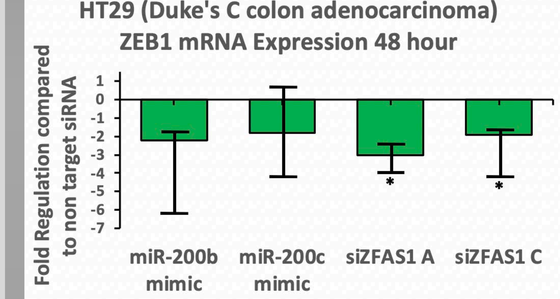


Figure 2)

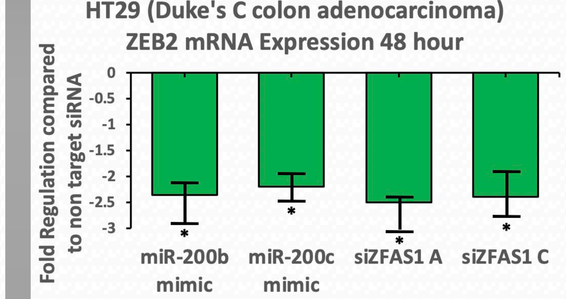


Figure 3)

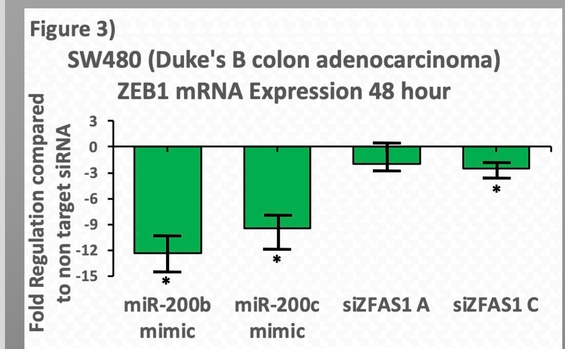
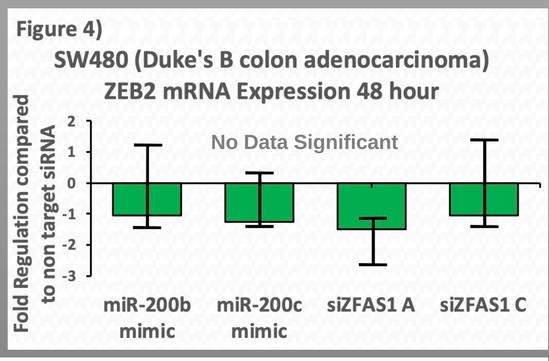


Figure 4)



***p<0.05, N=4, Median ± Interquartile range (Figures 1-4)**

- Successful transfection was confirmed.
- After transfection with siZFAS1A & C, HT29 cells showed decreased expression of ZEB1 and ZEB2 mRNA at 48 hours (p<0.05) (Figure 1,2).
- After transfection with miRNA-200b & c, HT29 cells showed decreased expression of ZEB2 at 48 hours (p<0.05) (Figure 2).
- Following siZFAS1C transfection, SW480 cells showed decreased expression of ZEB1 mRNA at 48 hours (p<0.05) (Figure 3).
- Following miRNA-200b & c transfection, SW480 cells showed decreased expression of ZEB1 mRNA at 48 hours (p<0.05) (Figure 3).

Conclusion

- The findings suggest that lncRNA ZFAS1 has an effect on the mRNA expression of ZEB1 and ZEB2 in the miRNA-200/ZEB pathway.
- Future goals are to delineate the in vitro effect of ZFAS1 expression on cellular phenotype.
- Further work is needed to evaluate the role of lncRNA ZFAS1 as a clinical target for the management of colon cancer.