

Introduction

- ❖ Stage III and IV colorectal cancer (CRC) are associated with poor prognosis and survival compared to earlier disease stages.
- ❖ Long non-coding RNAs (lncRNAs) have a role in regulating epithelial-to-mesenchymal transition (EMT) and tumor progression. lncRNAs have several mechanisms of action, one being a decoy for microRNA.
- ❖ ZFAS1 is a lncRNA that is upregulated in CRC and has been shown to interact with the miR-200 family.
- ❖ The miR-200 family represses EMT, and is associated with more favorable prognosis.
- ❖ We hypothesized that ZFAS1 knockdown would lead to an increase in the expression of the miR-200 family.
- ❖ Additionally, we studied if transfection with miR-200 family mimics would lead to decreased expression of ZFAS1.

Methods

- ❖ Two CRC cell lines (SW480 and HT29, Duke's B and Duke's C, respectively) were grown in culture medium until confluent.
- ❖ Cells were plated into separate wells at a concentration of 1.6×10^5 cells/well, and allowed to adhere for 24 hrs.
- ❖ The cells were then transfected with a ZFAS1 silencing RNA (siRNA), along with miR-200b mimics, miR-200c mimics, or negative controls.
- ❖ RNA was extracted at 24, 48, and 72 hrs post-transfection.
- ❖ Reverse transcription and quantitative Real-Time Polymerase Chain Reaction were performed to determine the expression of miR-200b, miR-200c, and ZFAS1.

Results

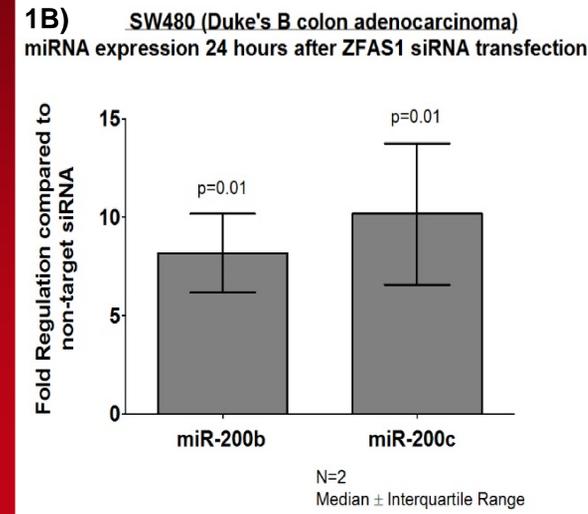
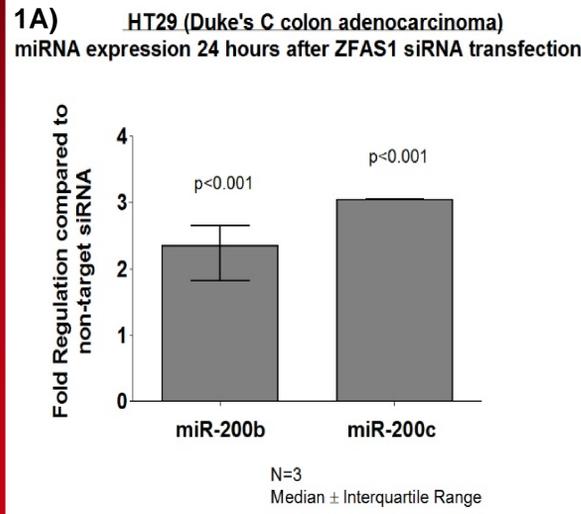


Fig. 1: (A-F) Results are in comparison to respective non-targeting siRNA with a fold regulation normalized to 1. **Fig. 1: (A)** Following ZFAS1 siRNA transfection in CRC cells in the HT29 (Duke's C colon adenocarcinoma) cell line, both miR-200b and 200c showed increased expression at 24 hrs post-transfection (Fold change=2.35, $p < 0.001$, and Fold change= 3.03, $p < 0.001$, respectively). **Fig. 1: (B)** miR-200b and 200c expression was also significantly increased in the SW480 (Duke's B colon adenocarcinoma) cell line at 24 hrs post-transfection (Fold change= 8.17, $p = 0.01$, and Fold change= 10.2, $p = 0.01$, respectively).

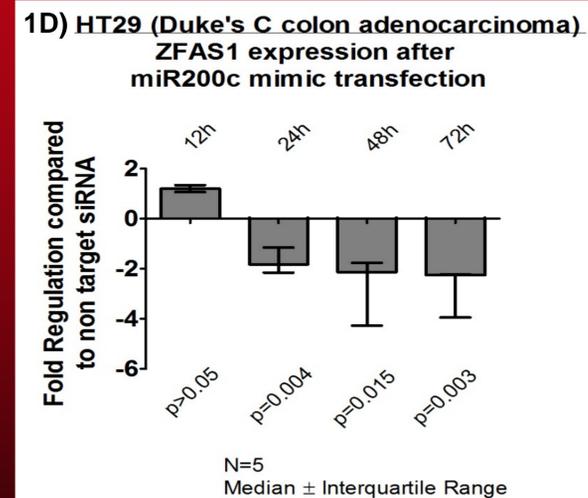
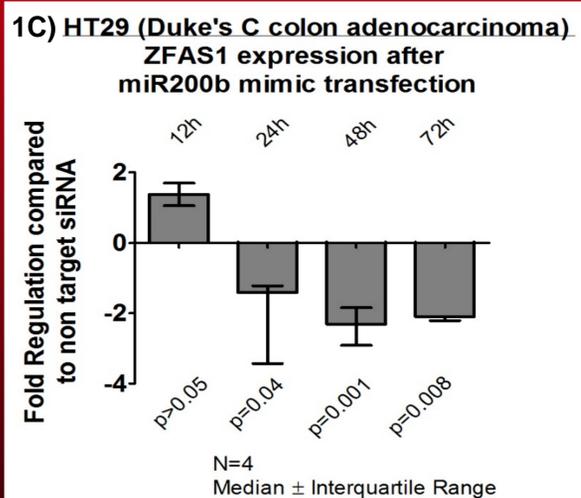


Fig. 1: (C&D) Following transfection of miR-200b and 200c mimics in the HT29 cell line, ZFAS1 expression was significantly decreased at 24, 48, and 72 hrs ($p < 0.05$) post-transfection. No significant fold change regulation was observed at 12 hrs post-transfection with miR-200b and 200c mimics in the HT29 cell line ($p > 0.05$).

Results (continued)

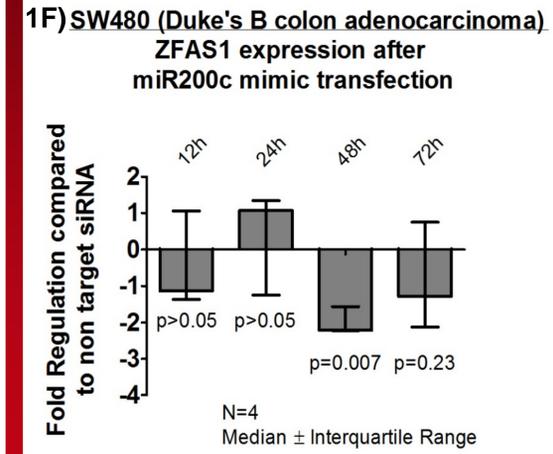
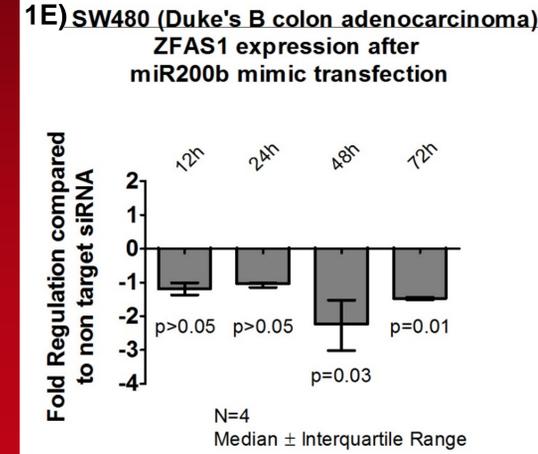


Fig. 1 (E) Following transfection of miR-200b mimics in the SW480 cell line, ZFAS1 was significantly decreased at 48, and 72 hrs ($p < 0.05$); however, no significant fold change regulation was observed at 12 or 24 hrs post-transfection ($p > 0.05$). **Fig. 1 (F)** Following transfection of miR-200c mimics in the SW480 cell line, ZFAS1 was also significantly decreased at 48 hrs, however, no significant fold change regulation was observed at 12, 24, or 72 hrs post-transfection ($p > 0.05$). P values were determined using an unpaired t-test and results were considered significant when $p < 0.05$.

Conclusions

- ❖ As hypothesized, knockdown of ZFAS1 leads to an increase in the expression of the miR-200 family in both cell lines.
- ❖ Similarly, transfection with miR-200 family mimics leads to decreased expression of ZFAS1, as hypothesized.
- ❖ These findings suggest that ZFAS1 has a direct relationship with the miR-200 family.
- ❖ ZFAS1 may facilitate tumor progression by inhibiting expression of the miRNA-200 family, therefore blocking the inhibition of EMT.
- ❖ ZFAS1 must be further investigated as a potential target in the treatment of CRC.

Acknowledgements

Research supported by a grant from the National Cancer Institute Grant R25-CA134283 and the John Williamson and Barbara Thruston Atwood Price Trust.