Effect of Long Non-Coding RNA ZFAS1 on Epithelial-To-Mesenchymal Transition Protein Expression in Colorectal Cancer Cell Lines

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Introduction

- Colorectal cancer (CRC) is the second most common cause of cancer-related deaths in the United States.
- Epithelial-to-mesenchymal-transition (EMT) is a major process involved in CRC whereby cells undergo cellular reprogramming and acquire a mesenchymal phenotype.
- EMT activation represses cell adhesion proteins, such as E-cadherin, and increases expression of mesenchymal proteins, such as vimentin.
- Long non-coding RNAs (lncRNA) are mediators of cancer signaling and affect gene expression.
- The lncRNA ZFAS1 is increased in colorectal adenocarcinomas and is associated with decreased overall survival.
- Previous studies have shown that ZFAS1 may have a role in activating EMT in various cancers.
- We hypothesize that silencing ZFAS1 will alter EMT protein expression in two well-studied colon adenocarcinoma cell lines.

Methods

- HT29 (Duke's C) and SW480 (Duke's B) cell lines were plated in 6-well plates at a concentration of $1.6x10^5$ cells/well and allowed to adhere for 24 hours.
- Cells were transfected with small interfering RNAs (siRNA) for ZFAS1 knockdown or a non-target negative control for 24 hours.
- Successful transfection was confirmed with qRT-PCR.
- Cell lysates were harvested and lysed using RIPA buffer. Total protein concentration was determined using a bicinchoninic acid (BCA) assay.
- Forty micrograms of protein were loaded into a 4-12% bis-tris gel electrophoresis.
- E-cadherin, vimentin and beta-actin proteins were probed using specific primary antibodies.

Methods continued

- E-cadherin and vimentin proteins were normalized to beta-actin housekeeping protein to obtain relative density units of E-cadherin and vimentin.
- Statistical analysis was performed using an unpaired t-test with significant results regarded as $p<0.05$.

Results

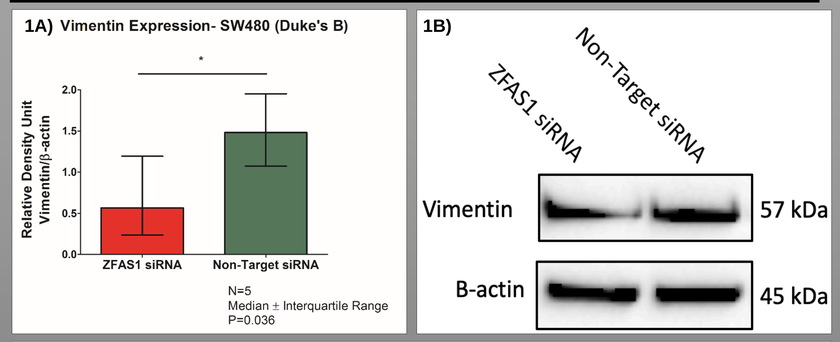

Fig. 1A-B: Vimentin expression significantly decreases in the SW480 cell line following ZFAS1 siRNA transfection

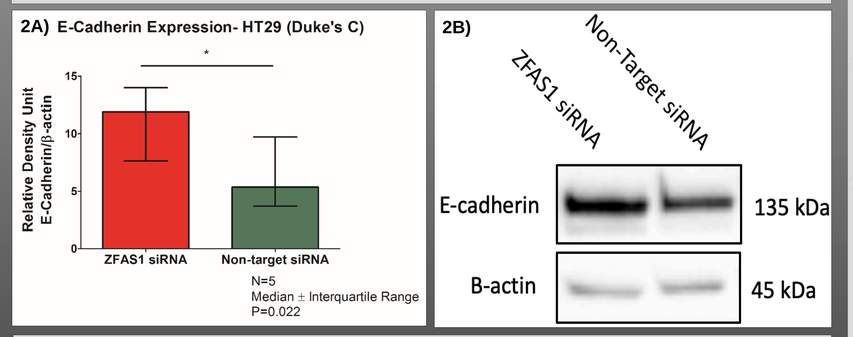

Fig. 2A-B: E-cadherin expression significantly increases in the HT29 cell line following ZFAS1 siRNA transfection

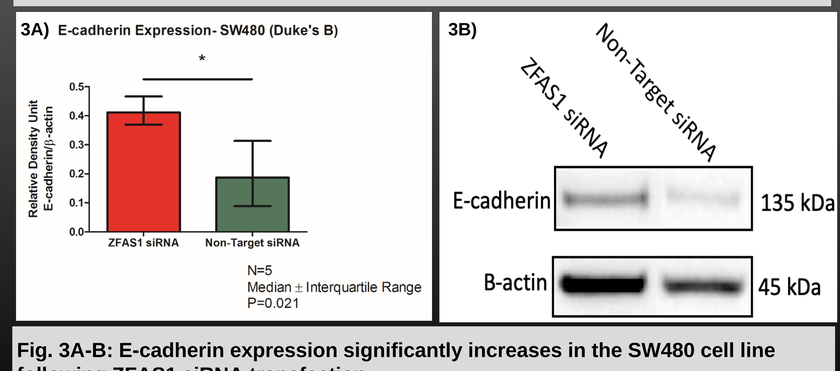

Fig. 3A-B: E-cadherin expression significantly increases in the SW480 cell line following ZFAS1 siRNA transfection

Results continued

- Following ZFAS1 siRNA transfection, the SW480 Duke's B cells showed a significant decrease in vimentin protein expression compared to non-target negative control ($p=0.036$) (Fig. 1A & 1B).
- After ZFAS1 siRNA transfection in the HT29 Duke's C cell line, E-cadherin showed a significant increase in expression compared to non-target negative control ($p=0.022$) (Fig 2A & 2B).
- Following ZFAS1 siRNA transfection in the SW480 Duke's B cell line, E-cadherin showed a significant increase in expression compared to non-target negative control ($p=0.021$) (Fig. 3A & 3B).

Conclusion

- Our findings show that lncRNA ZFAS1 has an effect on both vimentin and E-cadherin proteins involved in the EMT pathway.
- ZFAS1 knockdown led to a decrease in vimentin expression in the mesenchymal-like SW480 cell line, suggesting an ability to diminish metastatic potential.
- Additionally, ZFAS1 knockdown in the HT29 and SW480 cell lines led to subsequent increases in E-cadherin expression, suggesting that ZFAS1 silencing has the ability to restore the more favorable epithelial phenotype.
- These findings indicate that lncRNA ZFAS1 plays an important role in CRC progression from early to late stage disease and warrants further investigation.

Acknowledgments

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