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

James Burton, M.S.1,2, Miranda Schmidt, M.D.1,2, Stephen O’Brien, MB BCh BAO1, Susan Galandiuk, M.D.1

1Price Institute of Surgical Research, Hiram C. Polk Jr. MD Department of Surgery, Louisville, KY
2University of Louisville School of Medicine, Louisville, KY

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.

Results

- 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.

- 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 IncRNA 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 IncRNA ZFAS1 plays an important role in CRC progression from early to late stage disease and warrants further investigation.

Acknowledgments

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