Expression of Long Non-Coding RNA in Colon Adenocarcinoma and Matched Normal Adjacent Epithelium

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Introduction

- Colorectal Cancer (CRC) is the fourth leading cause of cancer-related death in the U.S. and is associated with significant morbidity.
- Long non-coding RNAs (lncRNAs) are large (>200 nucleotides) RNA molecules that make up a significant portion of the non-coding transcriptome and are important regulators of gene expression.
- We identified a panel of significantly upregulated lncRNAs through analysis of a colon adenocarcinoma RNA-seq data set.
- These lncRNAs have been associated with tumor progression and metastasis in a number of different kinds of cancer.
- Our aim was to validate the expression of these CRC-associated lncRNAs in fresh frozen CRC tissue and normal adjacent epithelium from patients undergoing initial surgical resection.

Methods

- Following informed consent, tissue specimens were taken from 8 patients with CRC (two from each stage) from the University of Louisville Surgical Biorepository.
- Tissue was cut and mounted on un-charged HistoBond+ ® glass slides.
- One slide per sample was stained with hematoxylin and eosin (H&E) to act as a guide for microdissection.
- Remaining fresh frozen tissue slides were stained and dehydrated using Arcturus® HistoGene® Frozen Section Staining Kit.
- Specific cells of interest were extracted with the ArcturusXT™ Laser Capture Microdissection System using H&E slides as reference.
- RNA was extracted and Isolated from tissue using the Arcturus® PicoPure® Frozen RNA Isolation Kit for LCM.
- Quantity and Quality of purified RNA was assessed using the NanoDrop2000™ (260/280).
- cDNA was generated through reverse transcription using SuperScript™ IV Vilo™ master mix.
- Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using lncRNA primers: FAM83H-AS1, PVT1, UCA1, H19, FER1L4, GAS5, ZFAS1, and GAPDH as an endogenous housekeeper.
- A two tailed paired t-test was used for analysis of cancer vs. normal and an unpaired one tailed t test was used for local vs. metastatic comparisons. Significance was defined as $p<0.05$.

Acknowledgements

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Results

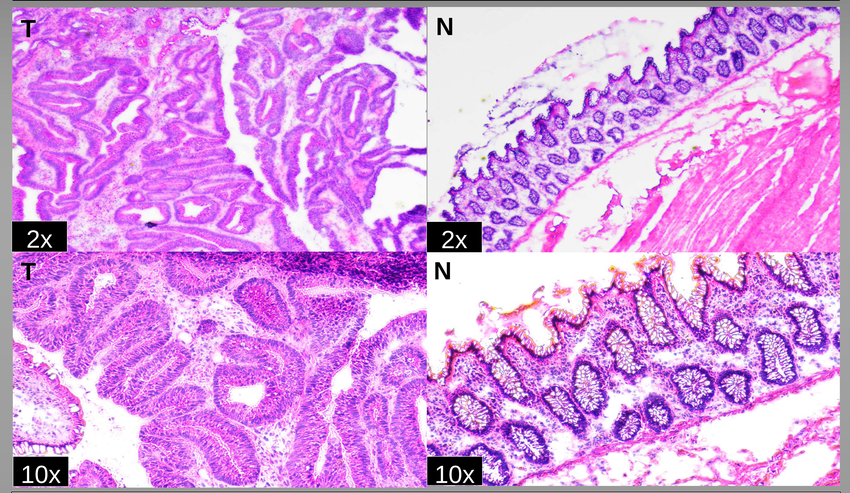


Fig. 1: H&E staining of malignant tumor tissue (T) and adjacent normal tissue (N) used as a guide in microdissection. In the tumor samples, only hypercellular, dysplastic tissue was excised. In normal epithelium samples, only normal epithelium was excised, leaving behind stroma and connective tissue matrix.

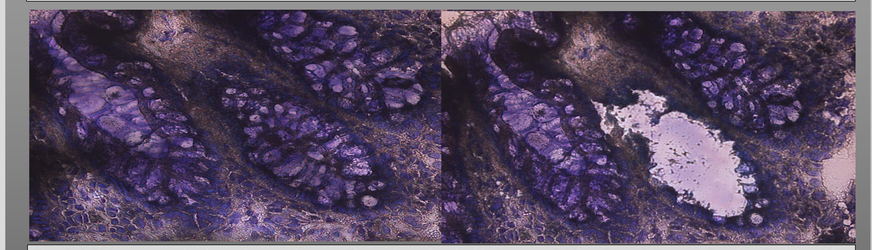


Fig. 2. LCM capture of colon adenocarinoma. Tissue is stained with Arcturus® HistoGene® Frozen Section Staining Kit. Dysplatic, hypercellular epithelium was selectively captured from the slide, leaving stroma behind.

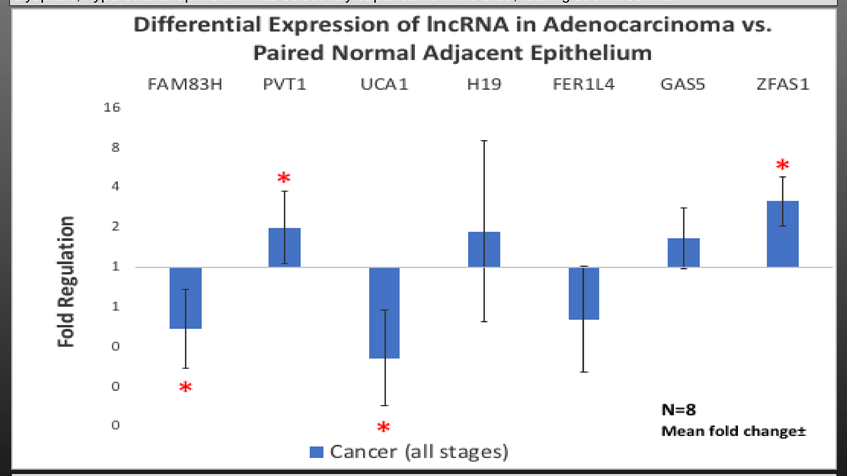
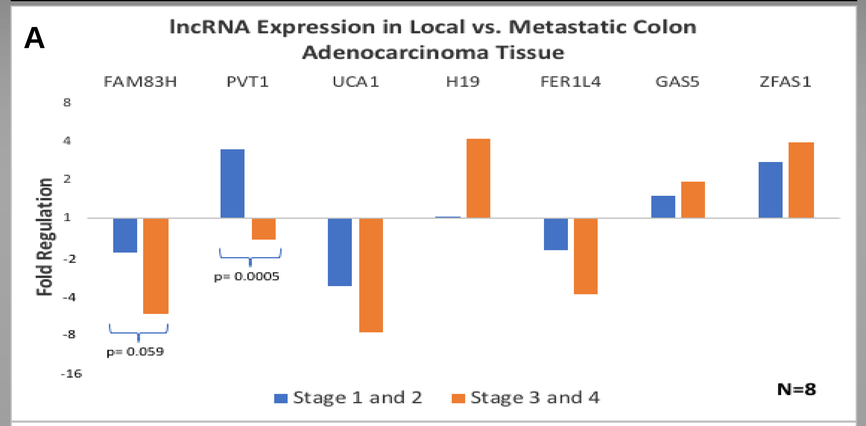


Fig. 3: **(A)** In CRC samples, PVT1 & ZFAS1 were significantly upregulated (FC=2.01, p=0.048 and FC= 3.17, p=0.0001, respectively) while FAM83H-AS1 & UCA1 were significantly downregulated (FC=-2.91, p=0.008 and FC=-4.85, 0.002, respectively) compared to normal adjacent epithelium.

Results cont.



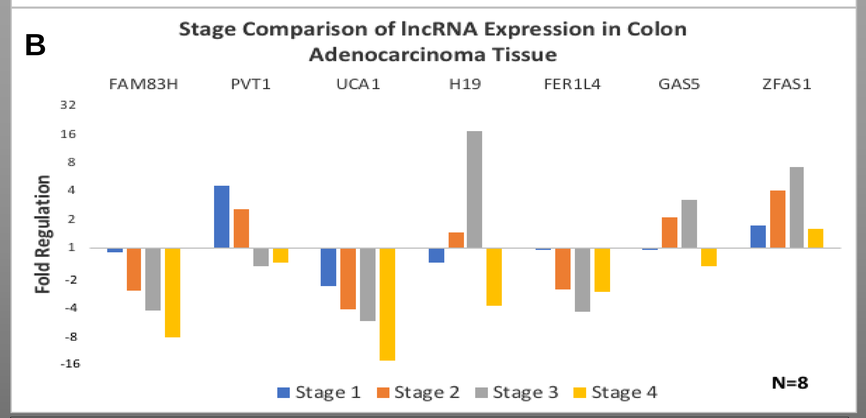


Fig. 4: **(A)** We observed a significant difference in the expression of PVT1 (p=0.0005) and a trend toward significance in FAM83H in local (Stage I & II) versus metastatic (Stage III & IV) expression (p=0.059). **(B)** No significant difference was observed between stages.

Conclusions

- PVT1 & ZFAS1 were shown to be significantly upregulated in CRC tissues while FAM83H-AS1 & UCA1 were significantly downregulated.
- PVT1 was also increased in local compared to metastatic cancers.
- These results validate the RNA-seq data set for the upregulated lncRNAs but are in contrast to the data set for downregulated lncRNAs.
- It is important to note our small study population and use of LCM tissue vs. whole tissue. We believe our data are more precise than previously published whole tissue data, which represents greater variability in expression profiles.
- Future studies will confirm observed expression profiles in CRC tissues and cell lines via PVT1 & ZFAS1 knockdown and FAM83H-AS1 & UCA1 knock-in experiments.