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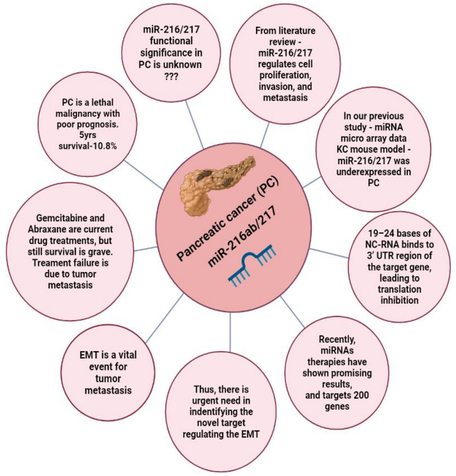
Abstract

Pancreatic cancer (PC) is a lethal malignancy with a 5-year survival rate of 10.8%. Gemcitabine, in combination with Abraxane and Folfirinox, are current treatments available for metastatic PC, however, all these therapies provided limited patient survival benefit, often resulting in high toxicity. Therefore, there is a need to identify novel therapeutic targets and combination therapies to combat this lethal cancer. MicroRNAs (miRNAs or miRs) have been shown to regulate PC proliferation and metastasis. Recently, miRNA therapies have shown promising results as a single miRNA is predicted to target more than 200 genes, involved in multiple pathways. The objective of this study is to understand the role of miR-216a/217 in PC growth and its progression. We and others have shown that miR-216a/217 was progressively downregulated during PC progression. PC patients with higher miR-216a/217 had better survival. Our *In-situ* hybridization data showed reduced expression of miR-216a/217 in PC patient samples (TMA - 196 core). Further, over-expression of miR-216a/217 in Capan-1 PC cells *in vitro* resulted in inhibition of cell proliferation and the epithelial-mesenchymal transition (EMT). *In silico* analysis have identified protein tyrosine phosphatase type IVA member 1 (PTP4A1) as a direct downstream target of miR-216a/217 in PC. Furthermore, our data indicates that miR-216a/217 inhibits PC metastasis by targeting PTP4A1 and may serve as a prospective therapeutic target in PC.

Background



Aim

The aim of the current study is to understand the role of miR-216a/217 in pancreatic cancer growth and its progression.

Results

Expression of miR-216a/217 in PC (GSE41369 data set)

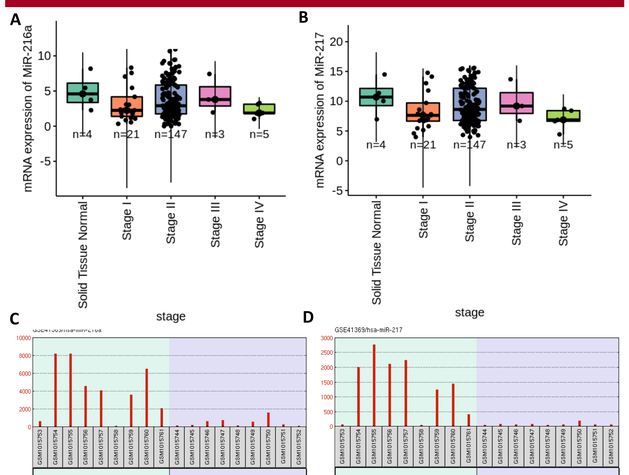


Fig 1. A&B. Expression of miR-216a/217 cluster during pancreatic cancer progression. **C&D.** miR-216a/217 expression in normal vs PC tissue.
Mean +/− SEM (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.001 n ≥ 3)

miR-216a/217 expression KC progression mouse model and human PC tissue

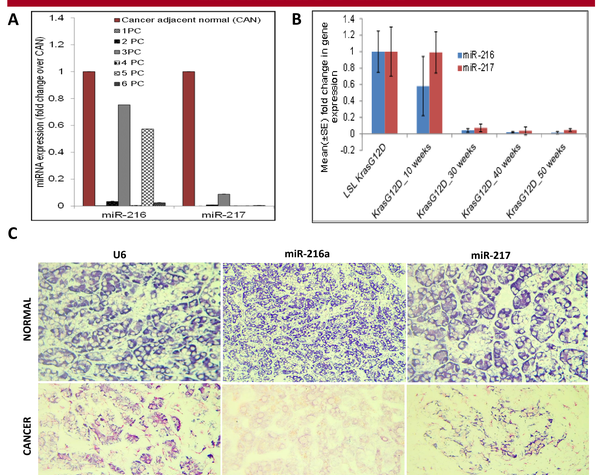


Fig 2. A. miR-216a/217 expression in human PC tissues samples **B.** miR-216a/217 expression pattern in KC progression mouse model. **C.** miR-216a/217 expression (*In-situ* hybridization) in tumor adjacent normal and PC tissues (TMA- 196 core). Mean +/− SEM (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.001 n ≥ 3)

Overexpression of miR-216a/217 reduced cell proliferation and EMT

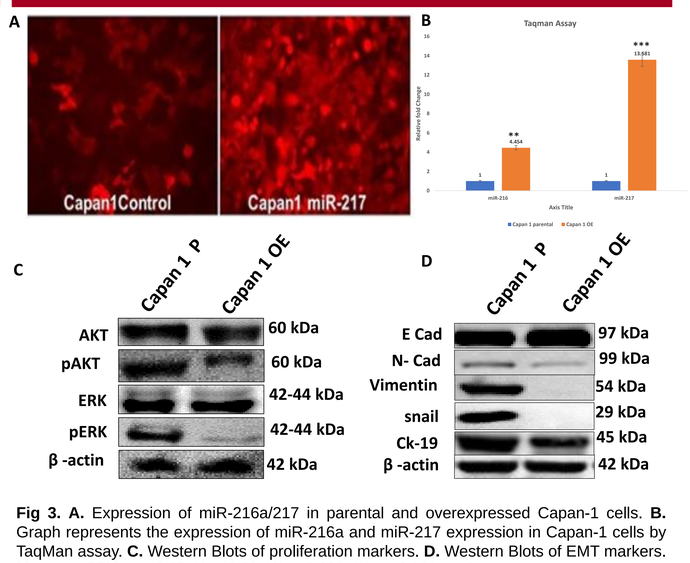


Fig 3. A. Expression of miR-216a/217 in parental and overexpressed Capan-1 cells. **B.** Graph represents the expression of miR-216a and miR-217 expression in Capan-1 cells by TaqMan assay. **C.** Western Blots of proliferation markers. **D.** Western Blots of EMT markers. Mean +/− SEM (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.001 n ≥ 3)

In-silico analysis for shortlisting the direct binding of gene involved in EMT

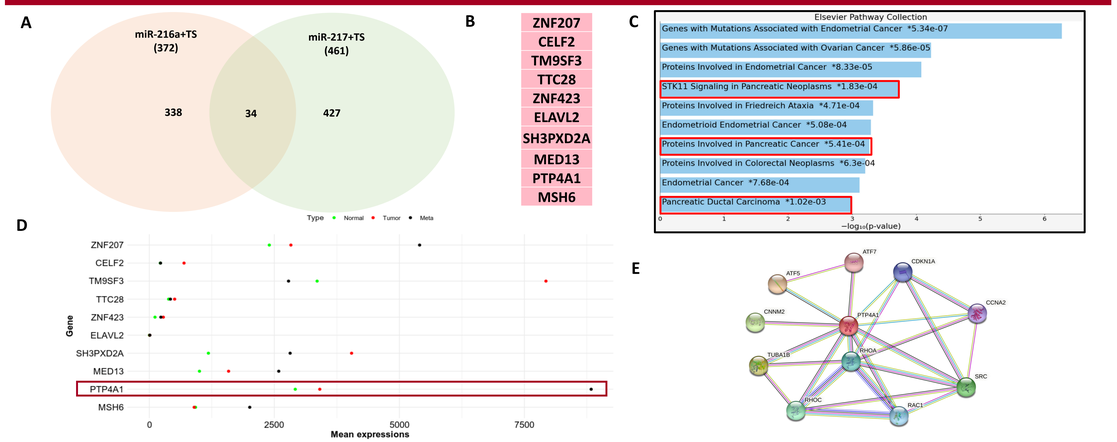


Fig 4. A. Venn diagram intersection shows list of genes that could be a direct target for miR-216a/217 **B.** Top 10 genes that could be direct target for miR-216a/217 **C.** List of pathways for top 10 genes involved. **D.** Expression levels of the top 10 genes in normal vs PC vs metastasis **E.** String analysis for PTP4A1

PTP4A1 could be a promising target for miR-216a/217

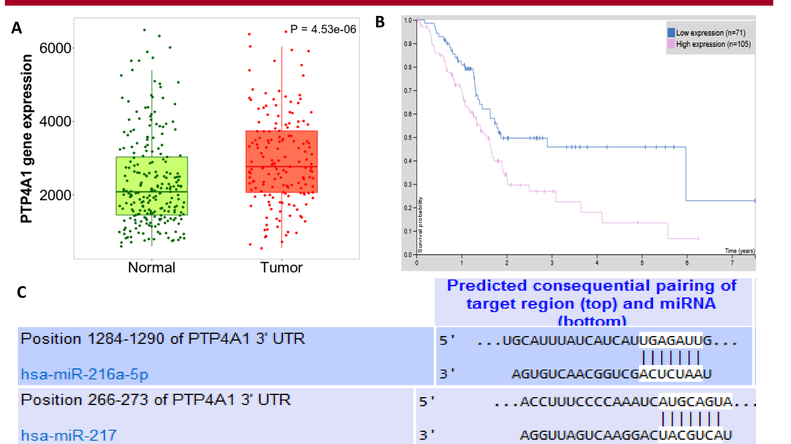


Fig 5. A. PTP4A1 expression in normal and tumorous tissue. **B.** PTP4A1 survival curve. **C.** target scan shows direct complementarity of miR-216a/217 and 3'UTR of PTP4A1.

Future Directions

- We will perform various *in vitro* and *in vivo* functional assays to assess miR-216a/217 role in proliferation, migration, invasion tumorigenicity and metastasis.
- RNA sequencing of PC cells overexpressing miR-216a/217 cluster to delineate the molecular mechanism(s).
- We will validate the direct binding of miR-216a/217 to the 3'UTR region of PTP4A1 gene by dual luciferase assay.
- siRNA of PTP4A1 in PC cell line and OE PTP4A1 w/o 3'UTR region of PTP4A1 in miR-216a/217 OE PC cells.

Conclusions

- miR-216a/217 are down regulated in PC.
- Restoration of miR-216a/217 showed reduced cell proliferation and EMT markers in pancreatic cancer.
- *In silico* analysis revealed that PTP4A1 could be a promising target gene via target-scan analysis.
- PTP4A1 showed increased expression in metastatic tumors suggesting that it could be an important target gene involved in EMT.
- Thus, restoring miR-216a/217 could be one of the potential therapies in PC.

References

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