The Effects of Ethanol on the Pancreatic Cell Line Transcriptomes

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Background

Pancreatic Ductal Adenocarcinoma (PDAC) is a highly aggressive cancer that develops from cells in the pancreas. Currently, PDAC has a 5-year survival rate of only 10% and it makes up about 7% of all cancer deaths (1). Certain risk factors are associated with PDAC development, including family history of cancer, obesity, diabetes, pancreatitis, alcohol consumption, and smoking. While several studies have assessed alcohol consumption and its contribution to PDAC development, there is conflicting evidence to whether or not alcohol actually promotes PDAC. Work from our lab indicates that specific subtypes of pancreatic cancer are associated with a patient's drinking status, which may influence treatment strategies and patient outcomes (2). This raises the question; How does alcohol affect cancerous and pre-cancerous pancreatic cells? In this study, we performed RNA-Sequencing on ethanol treated pancreatic cells in different stages of cancer progression to provide insight to the effects of ethanol on the etiology of this disease. We analyzed the protein coding genes that were differentially expressed between non-treated and ethanol treated cells and performed functional analysis to better understand the impact of ethanol on the biological processes in pancreatic cells.

Methods

ASPC1, HPNE-Kras, and HPNE cells were cultured in 100mM ethanol (EtOH) for 6 months. RNA Sequencing was performed and reads were mapped to the human genome. The data was normalized, and protein coding genes were used for differential expression analysis. We applied a fold-change threshold of 1.25 to ensure confidence in differentially expressed genes. The functional analysis of biological process GO terms were obtained through the software, ClueGO (3), by using protein coding genes that were common in the up- or down-regulated sets within the three EtOH cultured cell lines ASPC1 (ASPC1 EtOH), HPNE-Kras (HPNE-Kras EtOH), and HPNE (HPNE EtOH).

Comparison

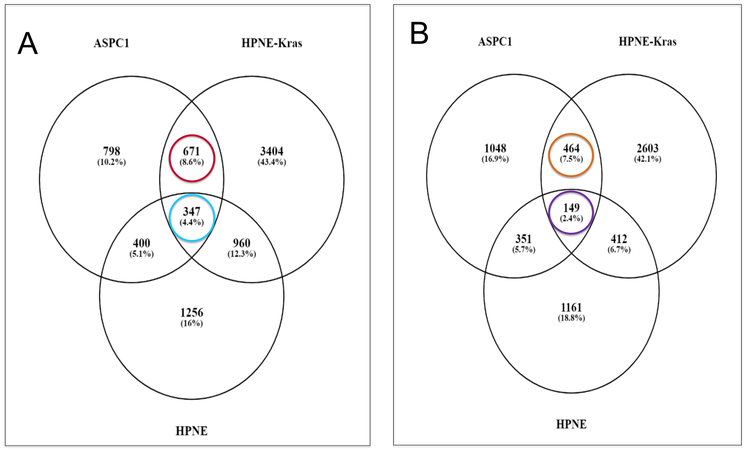

Fig 1. **A**. Venn diagram of up-regulated protein coding genes for ASPC1 EtOH, HPNE-Kras EtOH, and HPNE EtOH cell lines. **B**. Venn diagram of down-regulated protein coding genes for ASPC1 EtOH, HPNE-Kras EtOH, and HPNE EtOH cell lines.

Up-Regulated Biological Processes

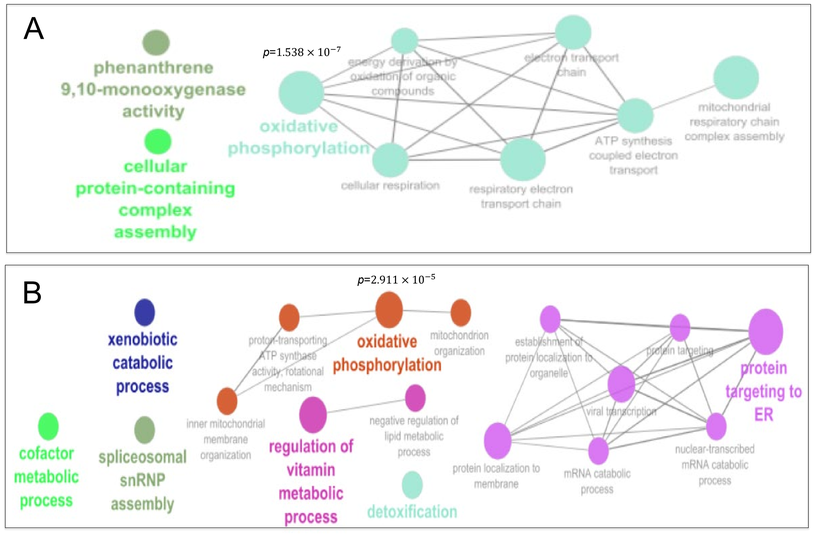

Fig 2. **A**. Gene Ontology of the genes up regulated in both ASPC1 EtOH and HPNE-Kras EtOH (Fig 1A red circle). **B**. Gene Ontology of the genes up regulated in all ASPC1 EtOH, HPNE-Kras EtOH, and HPNE EtOH cell lines (general effect of EtOH) (Fig 1A blue circle).

Down-Regulated Biological Processes

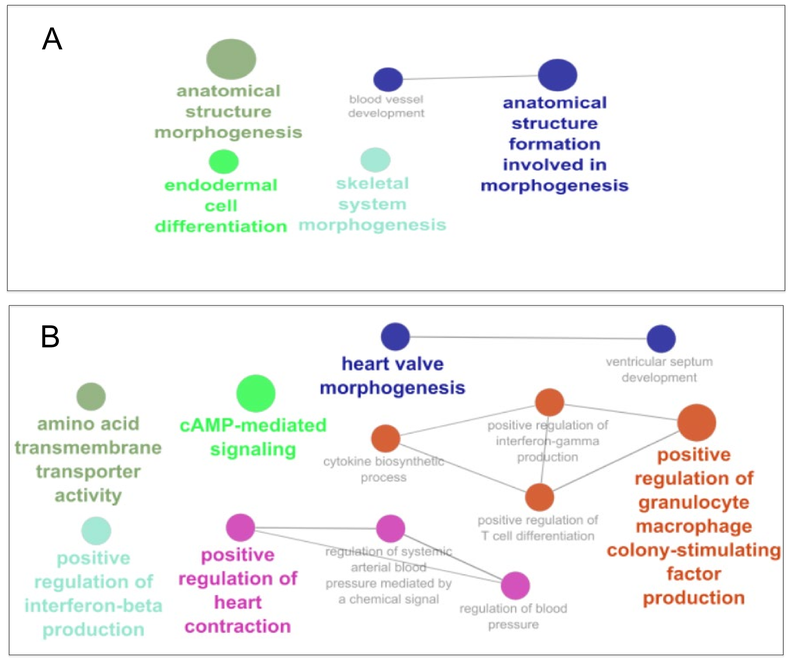

Fig 3. **A**. Gene Ontology of the genes down regulated in both ASPC1 EtOH and HPNE-Kras EtOH (Fig 1B orange circle). **B**. Gene Ontology of the genes down regulated in all ASPC1 EtOH, HPNE-Kras EtOH, and HPNE EtOH cell lines (general effect of EtOH) (Fig 1B purple circle).

Associated Genes

A

ATP5F1C	ATP5MC3	COX6A1	NDUFAF1	NDUFB3	NDUFB8	PARK7
ATP5MC2	ATP5PB	CYCS	NDUFB2			

B

ATP5F1B	ATPSCKM	COX7C	NDUFA2	NDUFB9	SDHAF2	UQCR11
ATP5ME	CCNB1	COX8A	NDUFA9	NDUFC2	SDHD	
ATP5PF	COA6	NDUFA11	NDUFB6	NUPR1	UQCC3	

Fig 4. Genes associated with the up regulation of oxidative phosphorylation in the general effect of EtOH(A) and Kras mutated cells (B).

Discussion and Conclusion

The functional analysis revealed the up-regulated protein coding genes promoted oxidative phosphorylation, protein targeting to ER, and regulation of vitamin metabolic processes. Oxidative phosphorylation was observed in both the ASPC1 EtOH/ HPNE Kras EtOH comparison and the upregulation of all cell lines treated with ethanol. However, when comparing the ASPC1 EtOH and HPNE-Kras EtOH figure (Fig 2A) to the general effect of EtOH in all three cell lines (Fig 2B), we can see that the oxidative phosphorylation is more significant in ASPC1 EtOH and HPNE-Kras EtOH. This suggests that functions associated with oxidative phosphorylation play a role in the general effect of ethanol, yet may play an even larger role when the cells become Kras mutated and/or fully transformed. EtOH is broken down in the liver and distributed to the rest of the body as acetyl-CoA. The cells then use the produced acetyl-CoA for their own metabolic process (4). The fact that the oxidative phosphorylation in Fig 2A is more significant could be caused by the need for the cancerous and pre-cancerous cells to perform an increase of cellular respiration to provide energy for their increase in proliferation.

For all cell lines treated with ethanol, downregulated functions associated with granuloctyte macrophage colony-stimulating factors, amino acid transport, INF-β and cAMP signaling were identified and specific to all three cell lines. These results may suggest a suppression of these factors as a general effect of ethanol treatment, while dysregulated morphogenesis may be more significant in the pre-cancerous and fully transformed cells. Previous research has suggested that abnormalities in morphogenesis promotes abnormal tissue formation and abnormal proliferation (5). The down regulation of anatomical structure morphogenesis found in ASPC1 EtOH and HPNE-Kras EtOH may be an indication for cancer morphogenesis occurring in the background. More research is required on a larger scale to further analyze the effects of ethanol on the transcriptome in pancreatic cells.

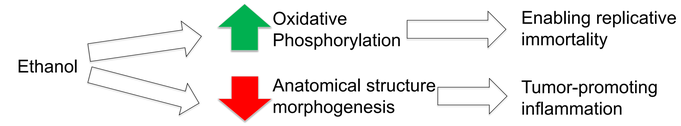

Fig 5. Ongoing hypothesizes to hallmarks of cancer.

Acknowledgments and References

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