Molecular characterisation of FFPE pancreatic tumours treated with 5-Fluorouracil (5-FU) and Sonodynamic Therapy (SDT) using whole transcriptome analysis

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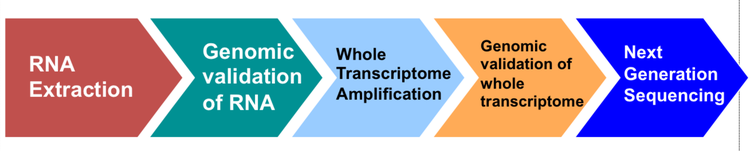
Introduction

•Current standards of care in pancreatic cancer (PC), such as surgical resection and chemoradiotherapy, remain ineffective in improving overall survival rates in PC

•Sonodynamic therapy (**SDT**) is a novel treatment modality that utilises ultrasound in conjunction with sonosensitisers to destroy tumors in a site-specific manner[1]

•This study aimed to investigate the effect of 5-Fluorouracil (**5-FU**) and SDT on expression levels of genes involved in aberrant signaling in PC using Next Generation Sequencing technology such as the Ion Proton™ System

Materials & Methods



•RNA extraction was performed on 3 FFPE specimens of BxPC-3 human pancreatic adenocarcinoma cells in a mouse model that were subjected to the following treatments[2]:

1.Untreated (**Control**)
2.440uM **5-FU**
3.O_2MB-RB* and 440uM 5-FU treated with ultrasound (**SDT**)

•Sample validation was performed using qRT-PCR, qPCR and a bioanalyser

•Whole transcriptome amplification was performed using an Ion AmpliSeq™ RNA Library Kit Whole transcriptome sequencing was performed using the Ion Proton™ System on amplified transcriptomes

** Oxygen-carrying microbubbles with covalently attached rose bengal on their surface*

•Bioinformatics analysis was performed using R/bioconductor and Database for Annotation, Visualization and Integrated Discovery (DAVID)

Results

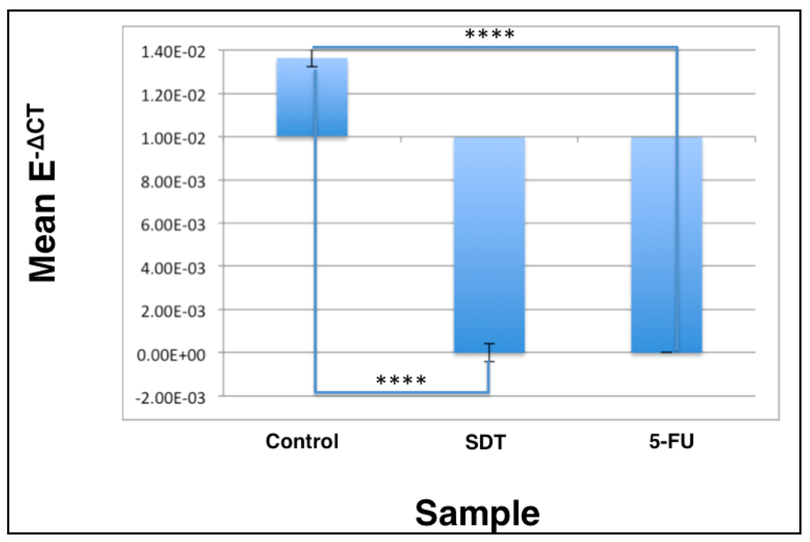


Figure 1. Mean $E^{-\Delta CT}$ of three samples. $E^{-\Delta CT}$ was calculated by taking $2^{-(\text{CT gene of interest} - \text{CT reference gene})}$ for each sample. Error bars represent standard error of the mean where n = 3

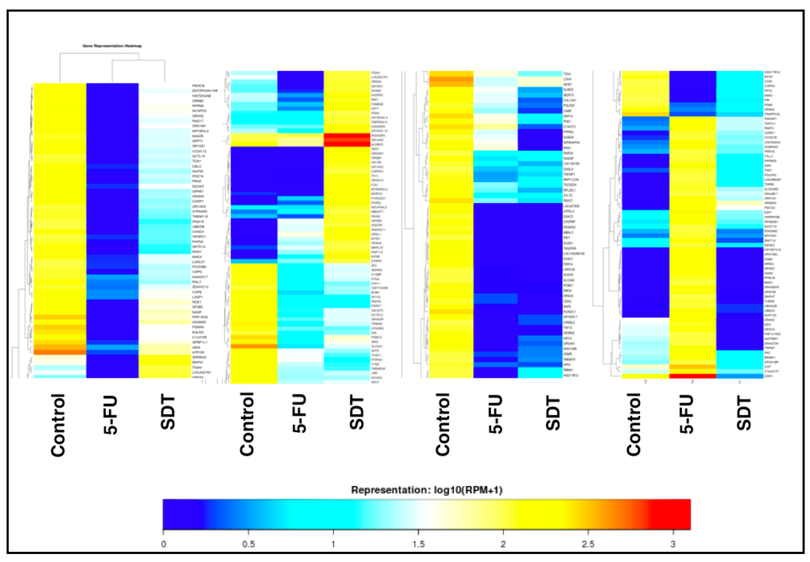


Figure 2. Unsupervised hierarchical clustering was applied to normalised RNA-seq values using R/Bioconductor. A heatmap representation of differentially expressed genes among all samples was generated

•Statistically significant differences in BCL3 expression levels between SDT and control (p = 0.001) and 5-FU and control (p = 0.001) were observed (Figure 1.)

•Clustering's heatmap shows different transcriptomic signatures between three transcripts suggesting that each treatment targets different transcriptomic signature (Figure 2.)

Discussion

•BCL3 expression was lower in both SDT and 5-FU treated samples as compared to untreated control sample, suggesting both treatment modalities cause lower BCL3 expression levels

•Functional clustering revealed the involvement of G-Protein coupled receptors (GPCR) and signal transduction pathways in PC

•Bioinformatics analysis also revealed two genes that showed the highest levels of differential expression between treated and untreated samples:

- **ATP1B1** had **8.94 times** lower expression levels in **5-FU** sample compared to **control**, and this plays an integral role in the membrane protein Na^+/K^+-ATPase involved in energy production[3]
- **RUNDC1** had **6.99 times** higher expression levels in **SDT** sample compared to **control**, and this is associated with a transcription factor that is involved with ubiquitination[4]

•Further work will validate the presence of ATP1B1 and RUNDC1 using qRT-PCR, by performing *in vitro* studies on untreated and treated cell lines

References

1.Trendowski. *Chemotherapy Research and Practice*. **2015**, 2015.
2.McEwan et. al. *Biotmaterials*. **2016**, 80.
3.National Centre for Biotechnology Information. **2016**.
4.STRING. **2016**.