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

Saif-U-Rehman Khan, Ryan Levi Seah, Rifat Hamoudi, Nikolitsa Nomikou

UCL Division of Surgery & Interventional Science

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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*

Results

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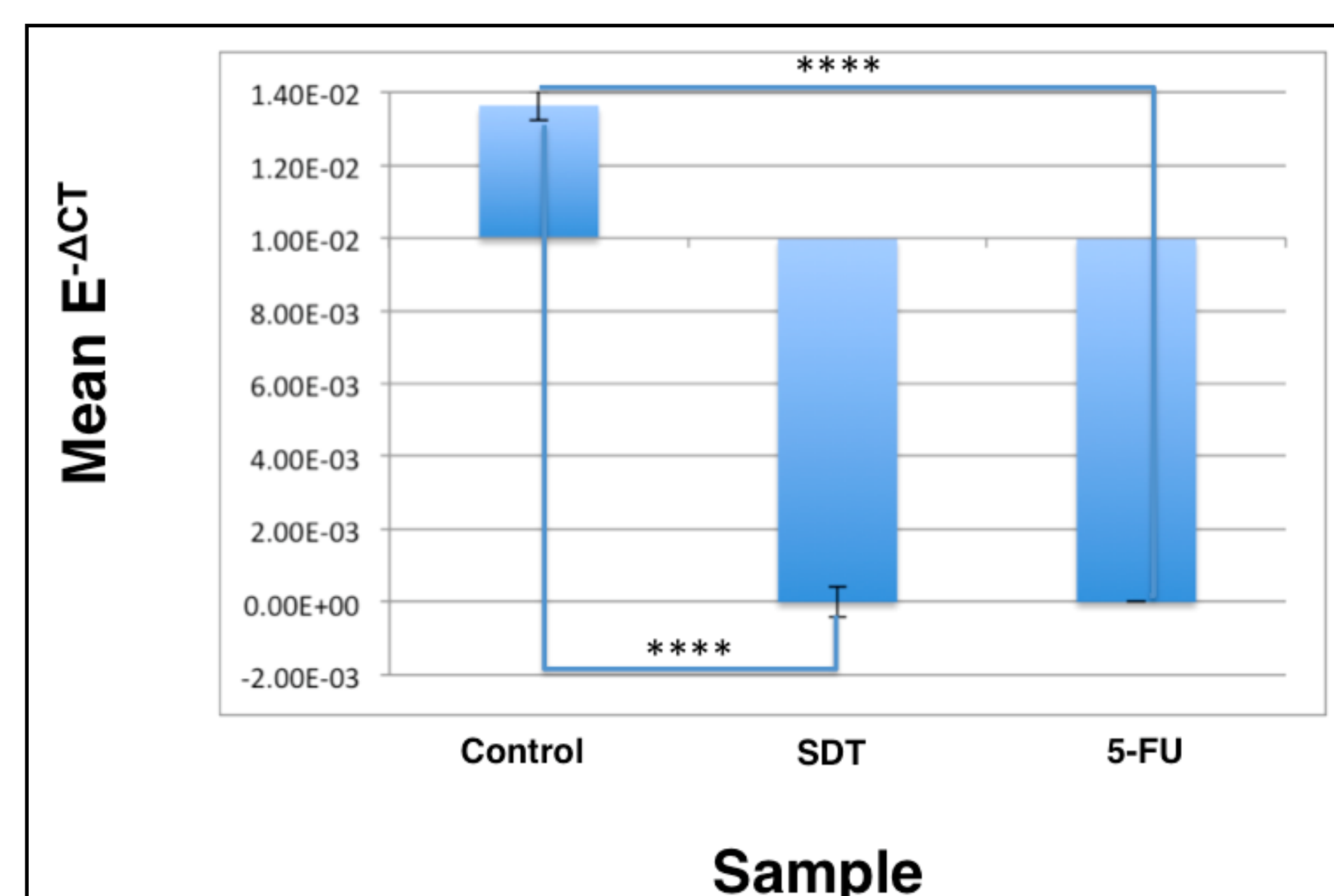

Figure 1. Mean $E^{-\Delta CT}$ of three samples. $E^{-\Delta CT}$ was calculated by taking $2^{-(CT\ gene\ of\ interest - CT\ reference\ gene)}$ for each sample. Error bars represent standard error of the mean where n = 3
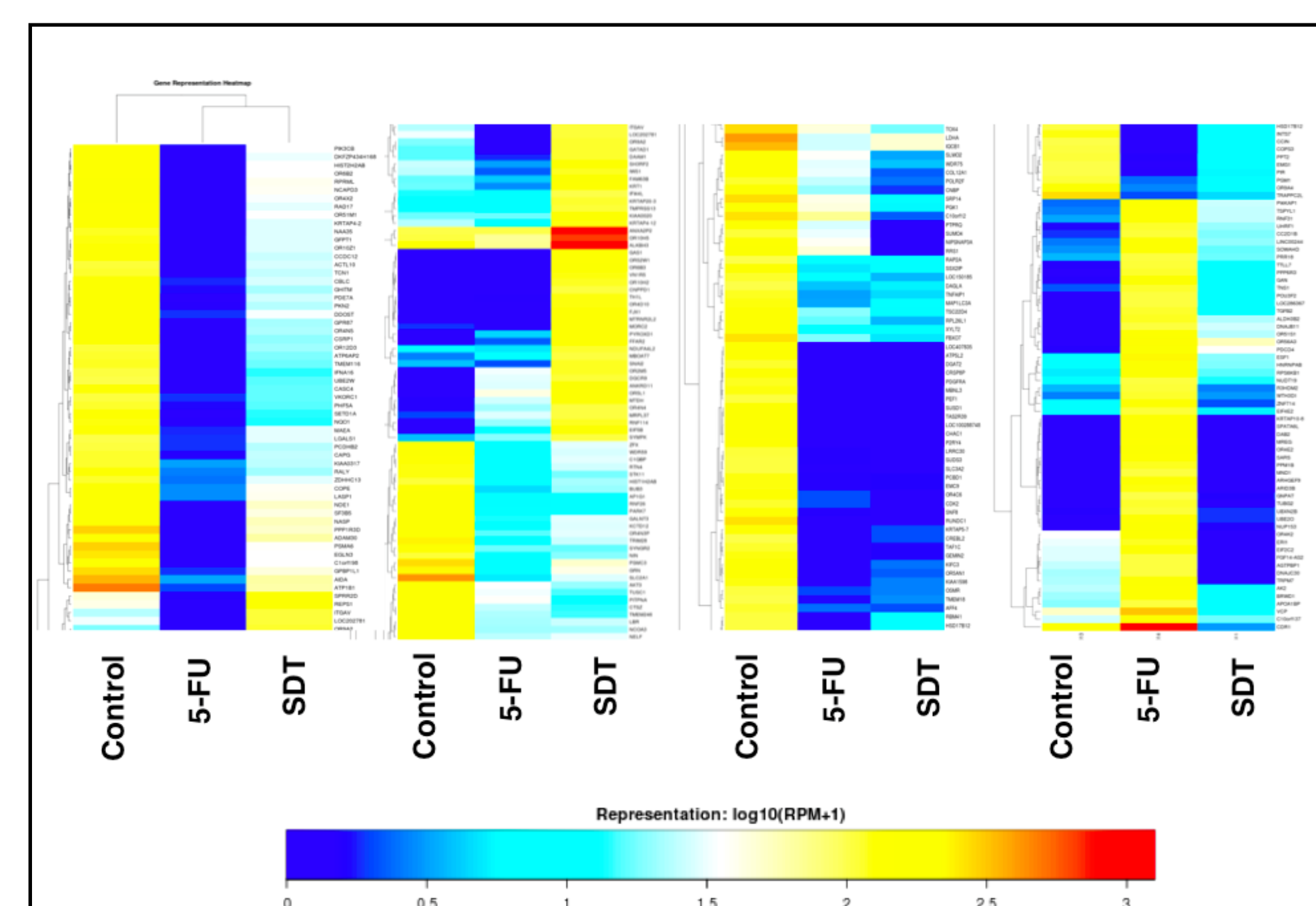

Figure 2. Unsupervised hierarchical clustering was applied to normalised RNA-seq values using R/Bioconductor. A heatmap representation of differentially expressed genes among all samples was generated

• 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**.