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.
• 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:
  1. Untreated (Control)
  2. 2440uM 5-FU
  3. O₂MB-RB* and 440uM 5-FU treated with ultrasound (SDT)

• Sample validation was performed using qRT-PCR, qPCR and a bioanalyzer.
• 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 \( 2^{(-CT \text{ gene of interest} - CT \text{ reference gene})} \) 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.
  • 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.

• 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

4. STRING. 2016.