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Molecular Pathways in Pancreatic Cancer Stem Cells

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease, with a 5-year survival rate of only 9%. One reason for this dismal prognosis is that PDAC is characterized by early and extremely aggressive metastatic spread. One of the most important hallmarks of metastatic capability is the acquisition and maintenance of a stem-like phenotype by cancer stem cells (CSCs), a process that is still poorly understood in PDAC.

AIMS

The main goal of this study was to identify enriched molecular pathways in pancreatic CSCs. For this purpose, we analyzed the transcriptional profile of established CSC-enriched tumorsphere cultures of PDAC patient-derived xenograft (PDX) tumors.

METHODS

- **Sequencing Data**: FASTQ files from adherent (non-CSC-enriched) and tumorsphere (CSC-enriched) cell cultures derived from 4 PDAC Patient Derived Xenografts (PDX) were obtained from *Sancho et al., 2015.*
- **Alignment to Human Genome**: Using STAR *2.604a*, the FASTQ files were aligned to Ensembl Human Genome GRCh38.105.
- **Transcript Abundance Estimation**: Using RSEM *1.3.1*, the *bam* files of aligned sequences had their respective count data exported into *tsv* files.
- **Differentially Expressed Genes**: To find DEGs, DESeq2 *1.30.0* R package was used, comparing the count data between the adherent and sphere culture samples of all patients. A patient-by-patient analysis was also performed.
- ***Survival Analysis***: Survival analysis was performed for DEGs using PDAC RNA-seq and clinical datasets from The Cancer Genome Atlas (TCGA; PAAD-US) (139 patients) and the International Cancer Genome Consortium (ICGC; PACA-AU) (90 patients).
- **Single Cell Analysis**: Violin plots were made using single cell RNA-seq data of 57,530 cells from 24 PDAC and 11 non-tumor pancreatic samples. These data were obtained from *Peng et al., 2019*

RESULTS

Heatmap of differentially expressed genes (DEGs) selected according to their fold change and statistical significance between adherent and tumorsphere expressions.

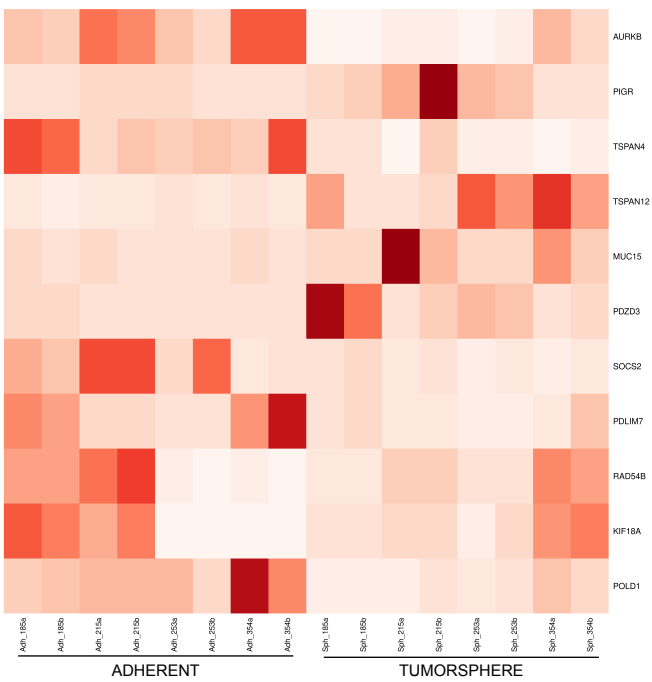

Figure 1. Heatmap comparing expression between adherent and tumorsphere cell cultures. 11 DEGs selected by their fold change and FDR value (q-value below 0.05). These genes are separated into Adherent samples (left) and tumorsphere samples (right).

To determine whether CSC-enriched DEGs are associated with PDAC prognosis, we correlated the expression level of each DEG with PDAC patient survival using publicly available TCGA (the Cancer Genome Atlas) and ICGC (International Cancer Genome Consortium) RNAseq and clinical datasets.

An inverse correlation between expression level in tumorspheres and survival was found for a subset of DEGs.

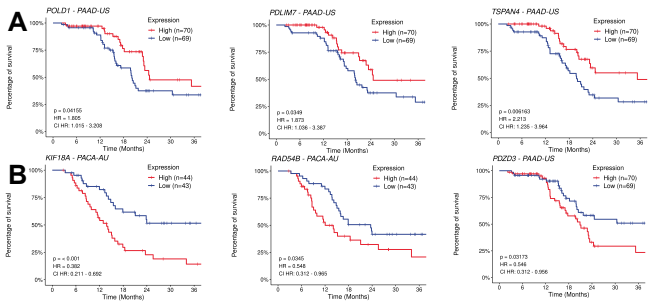

Figure 2. Kaplan-Meyer survival curves according to DEGs expression using public data consortia TCGA and ICGC. Patients were grouped into high and low expression groups for each DEG, according to the median expression. Kaplan-Meyer survival curves were generated using the R survival package. Survival curves were compared by the log-rank test with a significance threshold of $p < 0.05$. Panel A (above) shows DEGs enriched in PDX-derived adhrent cultures and Panel B shows DEGs enriched in PDX-derived tumorsphere cultures

In parallel, in order to correlate the expression of DEGs with the transformed phenotype in PDAC, we also investigated expression of the selected DEGs in PDAC tumors by analyzing single-cell RNAseq datasets. A subset of DEGs was enriched in type 2 ductal cells, which are found exclusively in PDAC tumors and represent the malignant cell population (Peng *et al.*, 2019).

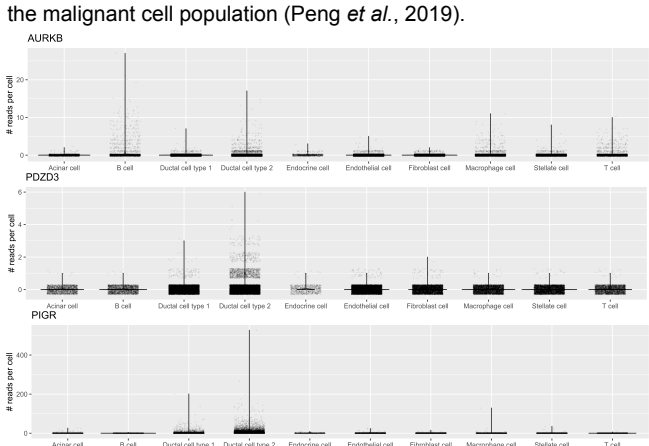

Figure 3. DEGs enriched in malignant type 2 PDAC cells. Violin plot of scRNA-seq analysis displaying normalized read counts (y axis) of AURKB, PDZD3, PIGR across 10 PDAC cell types (x axis) of 57,530 cells from 24 PDAC and 11 non-tumor pancreatic samples reported in Peng *et al.*, 2019.

It is noticeable that PDZD3 and PIGR are preferentially expressed in malignant ductal type 2 cells and AURKB is also highly expressed in this cell type. Considering that AURKB is a readily druggable therapeutic target studied in other types of cancer (Bausch-Fluck *et al.*, 2018), that high expression of PDZD3 is associated with worse PDAC survival, and that PIGR is a cell-surface protein, these DEGs represent promising therapeutic targets and/or biomarkers.

CONCLUSION

We have identified CSC-enriched DEGs that may represent potential therapeutic targets and potential novel biomarkers, that could be explored to improve PDAC therapy and prognosis. Future studies will aim to functionally validate the therapeutic and biomarker relevance of these targets.

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