Pancreatic heterospecies heterospheroids in cancer/stroma crosstalk investigation

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Background and Aim

Pancreatic ductal adenocarcinoma (PDAC) has by far the poorest outcome amongst solid cancers. The stroma plays a major role in tumor biology and treatment response. We developed a 3D in vitro cell culture model based on monospheroids/MS and heterospheroids/HS, which were made up from murine pancreatic stellate cells (mPSCs) and human pancreatic cancer cells (Panc1), in order to better understand the crosstalk between these two cell types.

Methods

Cells grown either as monospheroids/MS (mPSCs or Panc1 cells) or heterospheroids/HS (mPSCs plus Panc1 cells) in DMEM/F12 medium supplemented with 0,24% methyl cellulose, 10% FCS and antibiotics in non-coated 96-well plates. Five days after seeding, spheroids were collected and mRNA was extracted for RNA-sequencing. Different softwares were used to virtually separate transcripts in a species-specific manner (see Fig.1).

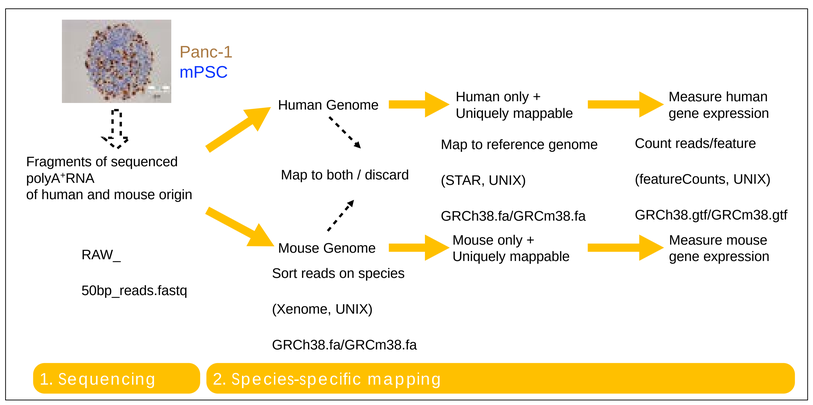

Fig1. Schematic description of species-specific expression analysis.

Results

We identified a total of 488 (660) differentially expressed protein coding genes with logFC >1 or logFC <-1 and FDR<0.05, consisting of 353 (225) downregulated and 135 (435) upregulated genes for the cancer cells (mPSCs) as a consequence of coculture with the stromal PSCs (Panc1 cancer cells).

GO terms of each category of the differentially expressed genes (logFC<-0.5 or >0.5) in Panc-1 (mPSCs) monospheroid and heterospheroid are shown in Fig.2 (FDR <0.05). Upregulated genes were enriched in cell division, extracellular matrix organization and cholesterol biosynthesis in Panc1 heterospheroid. For mPSCs, upregulated genes were enriched in Wnt signal pathway, Notch signal pathway, cholesterol biosynthesis and negative regulation of cell proliferation.

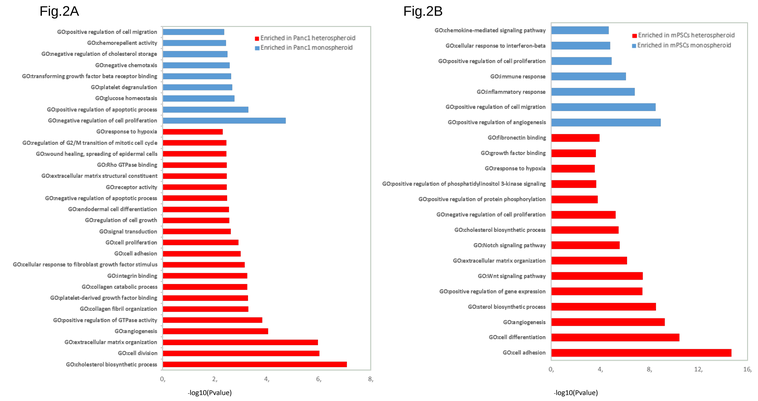

Fig. 2. GO enrichment analysis of Panc1 and mPSCs mRNAs, categories of each GO dataset in Panc1(A) and mPSCs(B) were shown.

The RNA-seq data were used to identify the classic and basal-like subtype signatures previously derived from bulk tissues following virtual microdissection (Moffitt et al. Nat Genet 2015). Panc-1 cells shifted from a classical/more differentiated phenotype to the basal-like/more aggressive phenotype upon co-culture with the mPSCs, while the mPSCs became activated by the co-cultured with Panc-1 cells (Fig. 3).

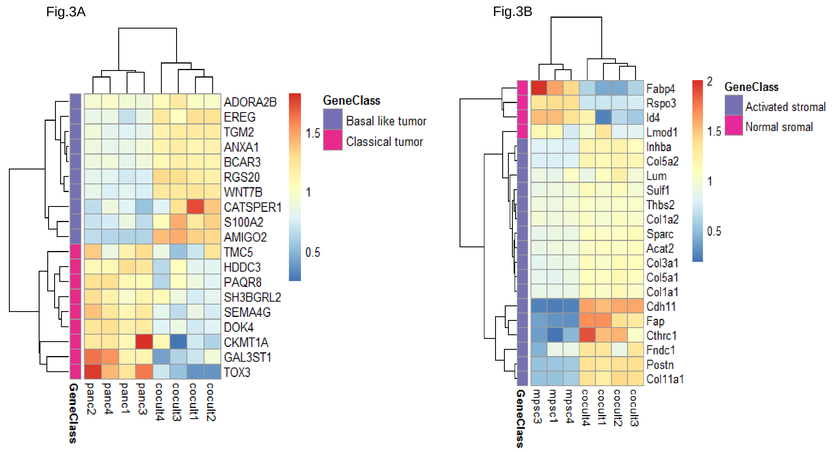

Fig3. Classification of Panc-1 cells in mono and heterospheroids according to the gene signatures for (A) basal-like and classical pancreatic cancer type. Also the mPSCs acquire a cancer associated fibroblast/stromal activated gene signature in the heterospheroid culture (B).

Conclusion and future directions

By virtual sorting of mRNAs in a species-specific manner, we are able to interrogate tumor-stromal crosstalk in mixed species PDAC heterospheroids.

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