Pancreatic stellate cells increase cancer cell expression of HMGA2 in a 3D co-culture model of pancreatic cancer

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Conclusion and future directions
Increased expression of TGFβ1 in the 3D co-cultures between pancreatic ductal epithelial (PDAC) cells and pancreatic stellate cells (PSCs) leads to an increased expression of HMGA2 in the cancer cells. We are currently investigating this mechanism in more detail.

Introduction
In a cohort of PDAC patients, positivity for the High Mobility Group AT–hook 2 (HMGA2) protein has been correlated to lower survival (Fig.1). One of the factors known to increase the expression of HMGA2 in other cancer cells, is Transforming Growth Factor-β1 (TGFβ1).

Aim
The aim of this study was to investigate the mechanism of increased HMGA2 in pancreatic cancer cells, and if this is mediated through interactions with stromal cells.

Methods
PDAC cells and PSCs were cultured in 3D, as mono- and co-cultures, in a newly developed model. Gene expression (mRNA) for cancer- and stellate cells within the co-culture model was investigated with real time PCR. This was done both for whole spheroids as well as for mono- and co-cultured spheroids sorted by Fluorescence Activated Cell Sorting (FACS). In order to look at cell type specific expression, Protein expression was determined by immunohistostchemistry (IHC).

Results
Expression of TGFβ1 mRNA increased in co-cultured vs mono-cultured PSCs in two separate tumor cell lines (Fig.2 A-B) HMGA2 was found to be higher expressed in co-cultured vs mono-cultured cells, both at the mRNA (Fig.2 A-B) and the protein level (Fig.2 C-E).

HMGA2 mRNA was also found to be higher specifically in the co-cultured vs mono-cultured Panc1 cells, in cells sorted by FACS (Fig.3 A-B). Further, TGFβ1 treatment of Panc1 mono-culture spheroids increased the expression of HMGA2 (Fig.3 B). Finally, we also compared TGFβ1 and HMGA2 basal expression levels in a panel of normal pancreatic and PDAC cell lines (Fig.3 C). We then identified a correlation between levels of TGFβ1 and HMGA2 in cell lines which were wild type for Smad4, a TGFβ1 downstream signaling molecule.

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