Pancreatic cancer/stroma cell cross-talk affects cell growth in a 3D spheroid co-culture model

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Conclusion and future directions
A pancreatic stellate cell (PSC) activation signature is expressed in early PSC-/tumor cell spheroid co-cultures. After day 3, PSC numbers are diminishing. Also tumor cell growth is affected by 3D co-culturing with PSCs. Co-culture causes an initial growth delay of Panc1 cells, whereas HPAFII cells have a significant growth advantage from 3D co-culture with PSCs.

Aims
The aim of this study was to validate a novel 3D spheroid co-culture model of pancreatic ductal adenocarcinoma (PDAC) cells and pancreatic stellate cells (PSCs), and to use said model as to investigate the effects of PDAC cell/PSC cross-talk on cell proliferation, cell death, migration and chemoresistance.

Methods
An eGFP-tagged PSC line and PDAC cell lines (Panc1 and HPAFII) were cultured in 3D mono- and co-cultures. Activation of the PSCs was investigated by real time PCR for whole spheroids and spheroids sorted by fluorescence activated cell sorting (FACS).

To investigate cell growth, cell numbers within the cultures were followed over time and the proportions of the different cell types within the co-cultures were determined by FACS.

3D spheroid mono- and co-cultures

Panc1
PSC
Panc1/PSC

Results
The mRNA-expression of the PSC activation marker α-smooth muscle actin (ASMA) as well as the extracellular matrix (ECM) proteins collagen I and lumican, the platelet-derived growth factor β-receptor (PDGFRB) and the transforming growth factor-β (TGF-β), downstream effector connective tissue growth factor (CTGF), were increased in PSCs within co-cultures as compared to mono-cultured PSCs (Figure 2).

PSCs are activated within the 3D co-cultures

PSCs were initially expanding but then diminished within 3D co-cultures independent of the co-cultured PDAC cell line (Panc1 or HPAFII, Figures 3 and 4). The presence of PSCs caused an initial growth delay of Panc1 cells in the co-cultures, but later a switch occurred, after which the Panc1 cells grew faster in co-culture than in mono-culture (Figure 3). Relative cell numbers of co-cultured Panc1 cells then caught up with that of the mono-cultured cells. This growth switch was preceded by a growth switch for the PSCs in the opposite direction. The proliferation of HPAFII cells, was on the other hand increased within co-cultures compared to mono-cultures (Figure 4).

3D co-culture of Panc1 and PSCs affects cell growth

3D co-culture of HPAFII and PSCs affects cell growth

We currently are investigating the effects on cell death and apoptosis, migration and chemoresistance.

Figure 1. Day 4 Panc1 and PSC 3D spheroid mono- and co-cultures.

Figure 2. Relative mRNA expression of indicated genes. MC = mono-culture, CC = co-culture.

Figure 3. Growth curve of 3D mono- and co-cultured cells as total cell numbers (A). Relative cell numbers compared to day 1 (B-D). Proportion of respective cell types within co-cultures (E-F). MC = mono-culture, CC = co-culture, numbers in () = ratio cells at seeding.

Figure 4. Growth curve of 3D mono- and co-cultured cells expressed as total cell numbers (A). Relative cell numbers compared to day 1 (B-D). Proportion of respective cell types within co-cultures (C). MC = mono-culture, CC = co-culture, numbers in parenthesis = proportion of cells at seeding.