Orthotopic transplantation of pancreatic cancer PDX models increases murine stroma content, but does not influence therapeutic response to standard of care

Ulrike Pfohl[1], Diana Behrens[1], Iduna Fichtner[1], Ole Daberkow[1], Wolfgang Walther[1,2] and Jens Hoffmann[1]

[1]EPO Experimental Pharmacology & Oncology Berlin-Buch GmbH, Robert-Rössle-Str., 10, 13125 Berlin, Germany, [2]Experimental and Clinical Research Center, Charité, Lindenberger Weg 80, 13125 Berlin, Germany

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Background

Pancreatic cancer remains a lethal disease with a 5-year survival rate of only 3 - 8 % after diagnosis of the tumor (WHO, 2012). Reasons for the poor prognosis are advanced and inoperable tumor stages at time of diagnosis and resistance to conventional therapies. One bottleneck in the development of novel therapies is the restricted availability of preclinical models. Patient-derived xenografts (PDX) represent a valuable tool for the prediction of therapie response, the identification of new biomarkers and therapeutic targets or pancreatic cancer specific activated pathways (MAPK, hedghog). However, PDX tumors differ from patient tumors as their surrounding tissue is replaced by murine stroma within few weeks after primary transplantation. Since the desmoplastic stroma has an impact on the progression and treatment of pancreatic cancer, we investigated the attributes of murine stroma components in PDX models of pancreatic ductal adenocarcinoma (PDAC).

Study outcome

1) Pancreatic PDX are characterized by a desmoplastic murine stroma due to distinct expression of murine collagen I and αSMA.

2) Steady expression of stroma markers demonstrate that stroma cells are not affected by therapy in these pancreatic PDX models.

3) Orthotopic PDX are considered to be the more clinical relevant models in comparison to subcutaneous PDX because they mimic the human pancreatic cancer microenvironment closely.

Methods

Xenotranplantation and passaging: Human tumor material was transplanted subcutaneously into immunodeficient mice within 24 h after surgery. Mice were visited twice weekly and observed for engraftment for a total time period of 4 months. Engrafted tumors were transplanted into new recipients when a tumor volume of approx. 1 cm^3 was reached in the primary passage.
Therapeutic characterization: After successful engraftment of the PDX (usually after passage 3 or 4), tumors were transplanted either subcutaneously or orthotopically into immunodeficient mice and a therapeutic study was initiated. Mice with advanced subcutaneous and orthotopic tumors were treated with different drugs relevant for the treatment of pancreatic carcinoma (used in the clinic as standard of care (SoC). Growth of orthotopic PDX models was monitored via high-resolution ultrasound. Tumor growth inhibition was evaluated in comparison to control (T/C) or according to modified RECIST criteria Tumors were preserved for stroma analysis 7 days after last treatment.
H&E staining / Immunohistochemistry: Paraffin-embedded xenograft material was stained with hematoxylin and eosin as described. Antibodies for staining of murine collagen I- and αSMA-positive cancer-associated fibroblasts (CAFs) were purchased from abcam and covalab, respectively.

Stroma fractions of orthotopic and subcutaneous pancreatic PDX (Panc12529) are not affected by standard therapy

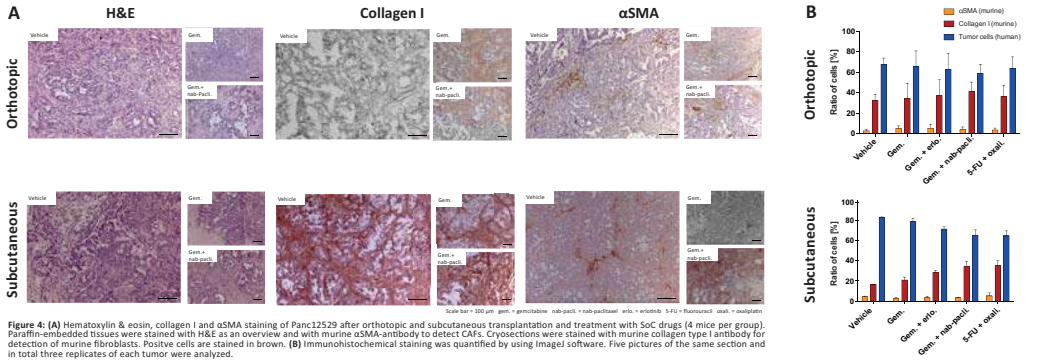

Figure 4: (A) Hematoxylin & eosin, collagen I and αSMA staining of Panc12529 after orthotopic and subcutaneous transplantation and treatment with SoC drugs (4 mice per group). Paraffin-embedded tissues were stained with H&E as an overview and with murine αSMA-antibody to detect CAFs. Cryosections were stained with murine collagen type I antibody for detection of murine fibroblasts. Positve cells are stained in brown. **(B)** Immunohistochemical staining was quantified by using ImageJ software. Five pictures of the same section and in total three replicates of each tumor were analyzed.

Semi-quantitative analysis of tumor sections showed that orthotopic tumors contain more murine stroma compared to subcutaneous tumors. SoC induced a reduction of tumor cell content, whereas the ratio of CAFs was not affected by SoC treatment.

Response to standard of care drugs of orthotopic and subcutaneous PDX tumors (Panc12650)

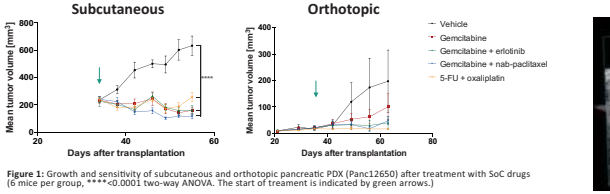

Figure 1: Growth and sensitivity of subcutaneous and orthotopic pancreatic PDX (Panc12650) after treatment with SoC drugs (6 mice per group, ****<0.0001 two-way ANOVA. The start of treament is indicated by green arrows.)

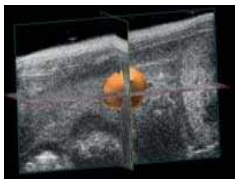

Figure 2: Three-dimensional ultrasound image of orthotopic Panc12650. Tumors were outlined and reconstructed into 3D to evaluate the tumor volume using Vevo*2100 software.

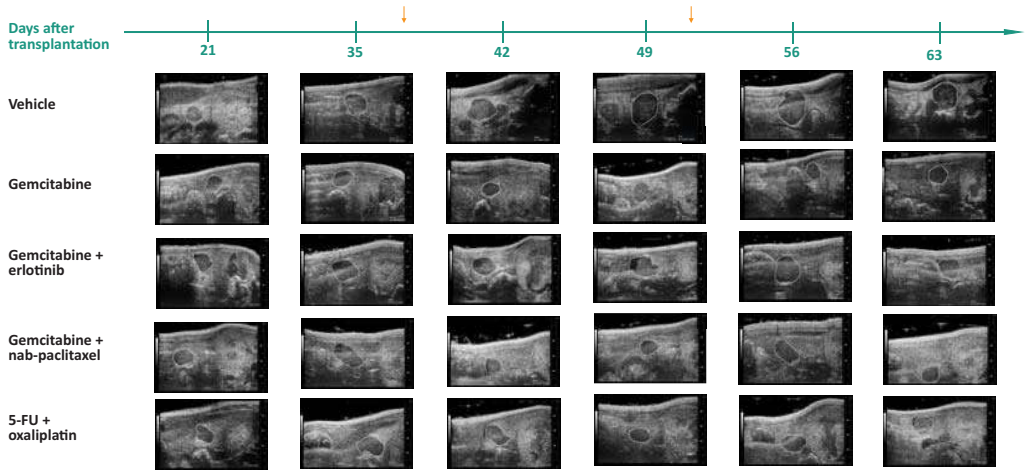

Figure 3: Monitoring of orthotpically growing Panc12650 before and after treatment with SoC drugs (6 mice per group). Figures show one representative tumor growth per treament group (Start and end of treatment areindicated by orange

In summary, there was an engraftment rate of Panc12650 of 100 %. The response profile in our experiments closly reflect patient's response in the clinic.

Conclusion

PDAC is characterized by a heterogeneous pheno- and genotype and represents a difficult-to-treat disease. This work reflects the current state of preclinical pancreatic cancer research and introduces concepts that contribute to the better understanding of the biology of pancreatic cancer and allow new, widely applicable therapy strategies. This study reveals that orthotopic transplantation results in xenografted patient tumors with an improved and functional tumor microenvironment. Tumor cells are more sensitive to therapy than CAFs. The remaining tumor stroma might provide a survival niche for some tumor cells leading to the frequently observed disease relapse. Further, targeting the tumor stroma might therefore be a promising approach to clinical treatmentof PDAC.

Outlook

There is growing interest in developing patient-derived xenografts for use in different cancer research applications. PDX have shown to be predictive of cinical outcomes for preclinical drug evalutation, biomarker identification and personalized medicine strategies.
A critical issue is the complete replacement of human stroma by murine stroma. Therefore, the humanization of the stroma or rather the establishment of pancreatic PDX with human CAFs would represent the patients situation better.

Corresponding address: jens.hoffmann@epo-berlin.com

www.epo-berlin.com