Analysis of murine stromal components in patient-derived xenografts (PDX) of pancreatic cancer

Diana Behrens[1], Ulrike Pfohl[1], Cora Hallas[2], Britta Büttner[1], Wolfgang Walther[1], Iduna Fichtner[3] and Jens Hoffmann[1]

[1]EPO Experimental Pharmacology and Oncology GmbH, 13125 Berlin-Buch, Robert Rössle Str.10, [2]Haematopathology Hamburg, 22547 Hamburg, Fangdieckstr. 75A
[3]Max Delbrueck Center for Molecular Medicine, 13125 Berlin-Buch, Robert-Rössle Str.10,

4080

Background

Pancreatic cancer remains a lethal disease with only 3 – 8% of patients surviving 5 years after diagnosis of the tumor (WHO, 2012). Reasons for this poor situation are advanced and inoperable tumor stages at time of diagnosis and resistance to conventional therapies. One bottleneck in the development of novel therapies of this disease is the restricted availability of preclinical models of high clinical relevance.
Since the desmoplastic stroma has impact on the progression and treatment of pancreatic cancer, we investigated the attributes of the murine stroma in patient-derived xenografts that completely replaced the human surrounding tissue within a few months after primary transplantation. We elucidated the functionality of the murine tumor microenvironment for growth and therapeutic response in a cohort of well-characterized pancreatic cancer (PDAC) PDX.

Study outcome

1) Patient derived xenografts (PDX) are characterized by a „desmoplastic" murine stroma due to distinct expression of murine α-SMA, Collagen I, FAP and SPARC

2) These murine stroma markers were not significantly affected by therapeutic intervention

3) PDX are well-defined preclinical tools for testing stroma-targeted treatment strategies

Panel of PDX models of pancreatic cancer

PDX	Gemcitabine	Gemcitabine + Abraxane	5FU + Oxaliplatin	Diagnosis	Mutation status
Panc_9699	-	+	-	pT3pN1, G3	KRAS, TP53, SMAD4
Panc_9996	-	++	-	pT3pN1, G3	KDR, MET, SRC
Panc_10713	++	++	-	pT4pN1, G3	KIT, KRAS
Panc_10991	+	+	-	pT3pN1, G3	KRAS
Panc_10953*	++	++	+	pT3pN1, G2	KRAS, TP53, SMAD4, KDR
Panc_11056	-	-	-	pT3pN1, G2	KRAS
Panc_11074	-	++	+	pT3pN0, G3	KRAS, TP53, SMAD4, KDR, CTNNB1
Panc_11159	-	+	-	pT3pN1, G3	KRAS, TP53, KDR, KIT
Panc_11344	+	+	-	pT3pN1, G3	KRAS, TP53
Panc_11495	+	++	+	pT3pN1, G3	KRAS
Panc_12529*	-	++	-	pT4pN1, G3	KRAS, TP53
Panc_12536*	-	++	-	pT4pN1, G3	Raf1 translocation

chosen for stroma analysis

Tab.1: Therapeutic sensitivity to SoC.
- no tumor growth inhibition (T/C > 50%)
+ moderate tumor growth inhibition (T/C 50-25%)
++ strong tumor growth inhibition (T/C < 25%)

Tumor sampling: Within the PAKANOSTRA project pancreatic cancer tissue was provided for xenotransplantation of corresponding clinics in Hamburg, Germany (cooperation within a frame work of a BMBF project). Tumor tissues were collected under sterile conditions and sent within 24 hours to the laboratories.

Methods

Xenotransplantation and passaging: The tumor samples were cut in small pieces under sterile conditions. One vital piece of the tumor was transplanted subcutaneously into the flank of immunodeficient mice. Mice were visited twice weekly and observed for engraftment for a total time period of 4 month. Engrafted tumors were transplanted into new recipients when a tumor volume of approx. 1cm³ was reached in the primary passage.

Therapeutic characterization: After successful engraftment of the PDX (usually starting from passage 3 or 4) a therapeutic study was initiated. 5 mice with advanced subcutaneous tumors per group were treated with different drugs relevant for the treatment of pancreatic carcinomas (used in the clinic as standard of care (SoC). Tumor growth inhibition was evaluated in comparison to control (T/C) or according to modified RECIST criteria. Tumors were conserved for stroma analysis 7 days after last treatment.

H&E staining/ Immunohistochemistry: Paraffin embedded tumor material/ xenograft material was stained with heamatoxylin and eosin as described. Antibodies for staining of murine Collagen I and α-SMA were purchased from Abcam and Covalab, respectively.

TaqMan RT-PCR Total xenograft RNA was isolated and processed according to suppliers protocols. TaqMan assay IDs: Mm00486332_m1 (SPARC), Mm01329177_m1 (FAP).

Mutation analysis: Illumina TruSeq Amplicon Cancer Panel.

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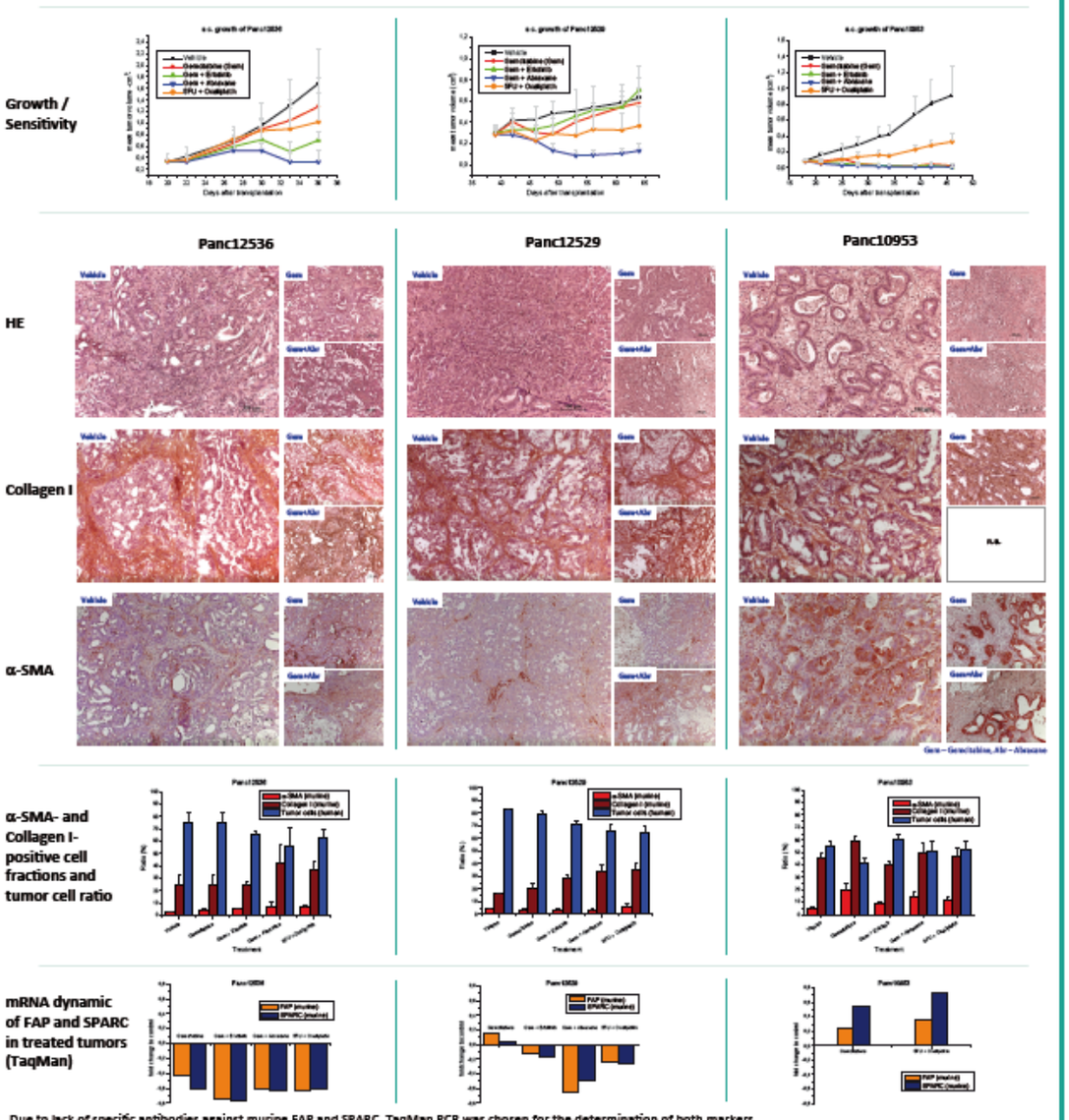
Expression of murine Collagen I, α-SMA, FAP and SPARC in a subset of subcutaneous PDX models



Due to lack of specific antibodies against murine FAP and SPARC, TaqMan PCR was chosen for the determination of both markers