Pancreatic cancer patient-derived xenograft (PDX) models: establishment and thorough molecular characterization

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Background

Pancreatic cancer (PC) is associated with poor prognosis, reflected by a high mortality and incidence rate. To overcome current insufficient treatment options for PC, we established a well characterized cohort of 45 PDAC PDX models with heterogeneous pheno- and genotype. Comprehensive information on genome data, mutational- and HLA status, morphology and in vivo growth as well as in vivo chemosensitivity towards standard of care drugs were compiled. This robust PDX panel allows the identification of biomarkers for treatment response and for new therapeutic vulnerabilities and broadens the spectrum of models of EPO.

Methods

- Xenotransplantation of patient-derived tumor tissues (PDAC: pancreatic ductal adeno carcinoma) was under sterile conditions and by using female NOG mice. Established PDXs were maintained in NRMI:nu/nu mice.
- Therapeutic characterisation was performed in NRMI:nu/nu mice using standard of care therapeutics (SoC) with optimized dosing.
- Data processing, mutational and gene expression analysis as well as HLA typing is published detailed: Behrens et al., Cancers (Basel). 2023;15(24):5753.

Results

- PDX models reflect patients characteristics in terms of growth, sensitivity to SoC and morphological appearance (Fig.1 and 2).
- Most frequent mutations in our PDX panel: KRAS, TP53, FAT1, KMT2D, MUC4, RNF213, ATR, MUC16, GNAS, RANBP2, and CDKN2A. This is similar to the frequency pattern that can be found in the relevant case cohorts hosted by the TCGA-GDC database (Fig. 3).
- Gene expression data identify signaling pathways (shown exemplarily for meaningful growth factors) and potential stromal responders (Fig. 4).
- PDX models show individual HLA class I and II gene expressions and cluster in different HLA sub classes (Fig.5).

PDAC PDX: In vivo growth and response to Standard of Care drugs

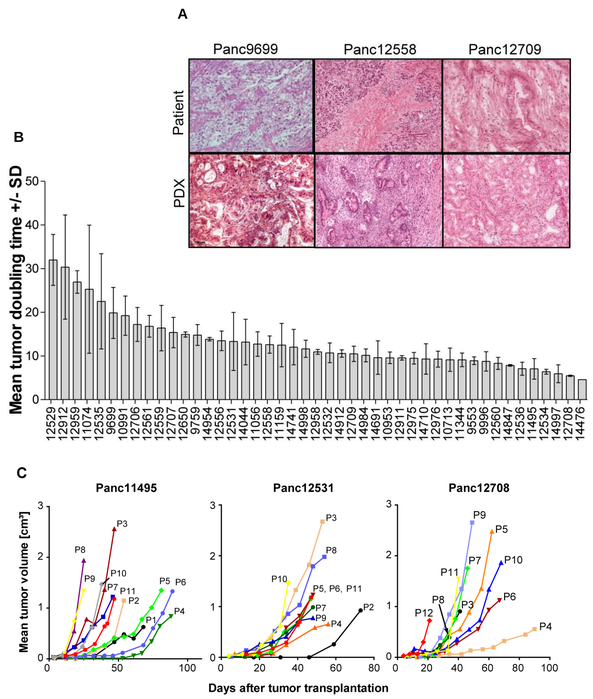

Figure 1: Histological features and growth characteristics of the PDAC PDX models. **A)** Comparison of the histology of the primary patient tumor tissues (upper panel) and of the corresponding PDX tissues (lower panel) in H&E stained tissue slices. Magnification: 40 ×. **B)** Mean tumor doubling times (TDT) of all 45 PDAC PDX models showing high range of the TDT for these models ranging from 5 days to up to > 30 days. Values represent means of 2 to 6 measurements with respective SD values. **C)** Alterations in TDTs shown for three representative PDAC PDX models during consecutive passages (from passage P1 up to passage P12), indicating accelerated PDX growth with increasing passage number on mice.

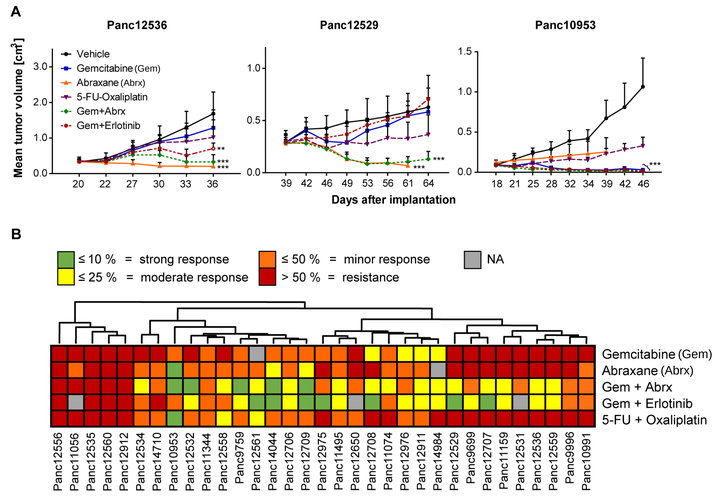

Figure 2: Sensitivity testing of PDAC PDX models toward SoC drugs in subcutaneous setting.
A) Mean tumor volume of s.c. PDX models during and after treatment with various SoC drugs (gemcitabine, abraxane, 5-FU, oxaliplatin) and one targeted drug (erlotinib), as well as drug combinations (gemcitabine/erlotinib; gemcitabine/abraxane; 5-FU/oxaliplatin). Tumor growth was monitored regularly from day 20 to 54 and the tumor volume was determined. Three representative PDX models (Panc12536, Panc12529 and Panc10953) with different level of sensitivity toward drug treatment are shown, reflected by changes of mean tumor volumes.
B) Unsupervised clustering of PDAC PDX models according to categorized T/C response rates of the individual treatment regimens showing sensitivity and resistance characteristics for all PDX models. ** p < 0.01, *** p < 0.001

Most frequent mutations in PDAC PDX

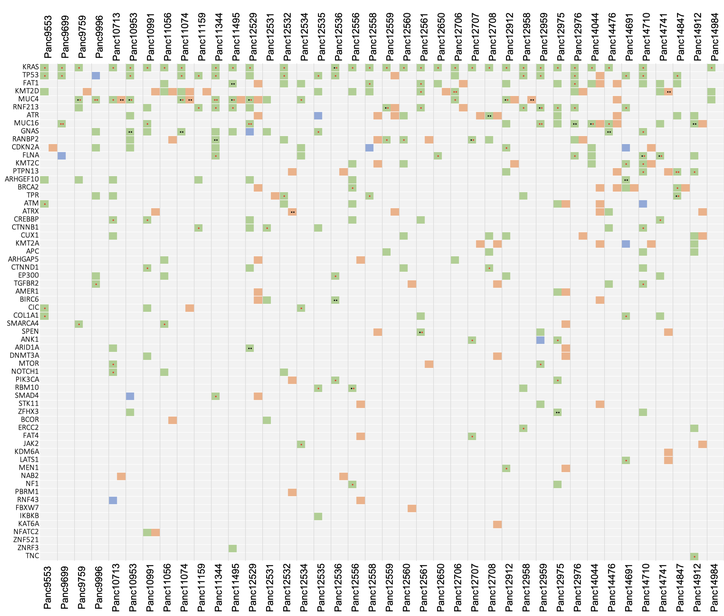

Figure 3: Mutational landscape of the pancreas carcinoma PDX cohort. Using variant calls from RNA sequencing data, 70 selected genes that are frequently mutated in the TCGA PDAC cohorts were analyzed for putative somatic mutations in 41 PDAC PDX models. We found putative somatic mutations in 62 different genes and in all 41 examined PDX models. Sequence variations were filtered based on population allele frequencies from the gnomAD database. Only variants that either were not included in gnomAD or have a gnomAD allele frequency below 0.05 were considered.

Gene expression profile (tumor:stroma interaction)

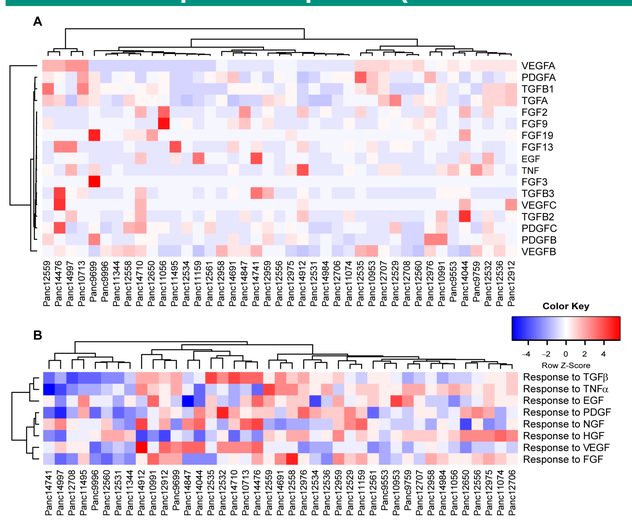

Figure 4: PDAC PDX models reflect the heterogeneity in tumor / stroma interactions.
(**A**) Unsupervised clustering of selected growth factor expression in tumor cells. Factors expressed below 10 TPM in all models were excluded from the analysis.
(**B**) PDX transcriptomes were scored for the enrichment of gene sets related to the cellular response of the indicated growth factors (GOBP, MsigDB) and hierarchically clustered.

HLA expression profiles of PDAC PDX

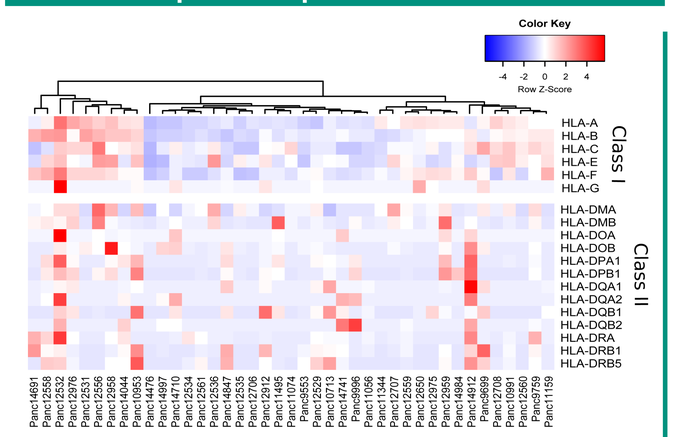

Figure 5: HLA profile of 41 PDAC PDX models. Unsupervised clustering of PDX models ac-cording to their gene expression (TPM) of HLA class I and II loci.

Conclusion

Our PDX panel represents the heterogeneous pheno- and genotype of PC in the clinic and can contribute to identify markers for progression and therapy response. The compilation of molecular and ECM information as well as immune related data provides a broad playing field for the development of novel drugs in a preclinical setup.

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