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Gastric cancer PDX models for predictive preclinical studies: Establishment, drug sensitivity, and genomic characterization

Michael Becker[1], Bernadette Brzezicha[1], Theresia Conrad[1], Jana Rolff[1], Michael Linnebacher[2], Christoph Treese[3], Beate Rau[4], Wolfgang Walther[1], Jens Hoffmann[1]

[1] Experimental Pharmacology & Oncology Berlin-Buch GmbH, Berlin, Germany; [2]Department of General, Visceral, Vascular and Transplant Surgery, University Medicine Rostock, Germany; [3]Division of Gastroenterology, Infectiology and Rheumatology, University Medicine Charité, Berlin, Germany; [4]Department of Surgery, University Medicine Charité, Berlin, Germany

Background and aim

Gastric cancer is a common malignant disease representing the fifth common cancer worldwide. Its prognosis is determined by the stage and localization (distal vs. proximal), making gastric cancer the third most lethal cancer type. In early and localized stages, the therapeutic aim is curative, in metastatic stages however, palliative treatment is the only option. Despite some progress in the last 10 years, mortality for the late stage gastric cancer is still high due to late diagnosis of the disease. We succeeded in establishing 7 patient-derived xenograft (PDX) models of gastric cancer that we are currently subjecting to a comprehensive characterization to determine their potential as a preclinical research tool for improvement of diagnosis and therapies.

Methods

For PDX establishment, tumor tissue from surgically resected gastric adenocarcinomas with different histologies was collected from consenting patients, implanted subcutaneously into immunodeficient mice and serially passaged for at least 3 *in vivo*-passages. The stable growing PDX were characterized towards sensitivity to the standard of care (SOC) drugs cisplatin, 5-FU, etoposid, epirubicin, docetaxel, oxaliplatin, and irinotecan. In parallel, we initialized a comprehensive molecular characterization starting with RNASeq analysis which was performed by Illumina® 80 Mio read paired-end sequencing of a TrueSeq® stranded mRNA library. Genome-wide gene expression was calculated from STAR-generated read counts using the *DESeq2* R-package. Expressed sequence variations were called using GATK4 and were interpreted by means of the Ensemble VEP.

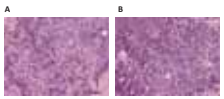
Results

We were able to establish a small set of 7 gastric adenocarcinoma PDX with different histologic patterns of a total of 90 clinical samples received, giving a take rate of 8%.

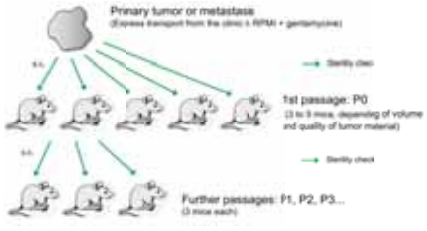
After treatment of PDX models with 7 standard chemotherapeutic drugs, individual response rates could be observed, with docetaxel giving the highest and cisplatin giving the lowest response rate. One model (Gast14171) turned out to be a relatively good responder, while another (Gast13765A) turned out to be worse.

A genome-wide gene expression analysis of RNASeq read counts revealed 3 distinct clusters that might reflect their histopathology. A deep analysis of expressed sequence variations found by the RNASeq variant calling resulted in 174 protein-changing mutations in 122 COSMIC-listed cancer-related genes; all of them annotated in SNPdb. Among these genes there are 5 putative cancer drivers carying truncating mutations as well as several genes associated to those described as mutated by the TCGA gastric cancer study (TCGA-STAD). 2 of the mutated drivers (***CDKN2A*** and ***TNFRSF14***) are already druggable. A parallel exome analysis is still ongoing.

PDX establishment

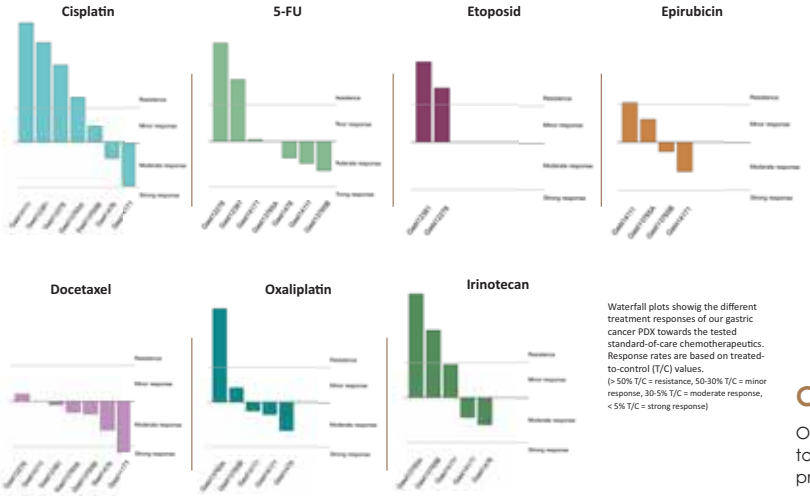


Gast13765B: The histology of the gastric cancer PDX remains stable after several passages.
(**A**) P1 (**B**) P7



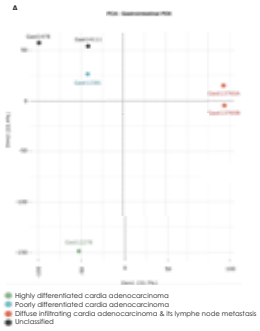
After surgery, pieces of patients tumor are express shipped and transplanted s.c. onto highly immunodeficient NOG mice immediately upon arrival. Several passages are performed until a stable growing human xenograft has developed. Tumors are maintained either *in vivo* by passaging or cryogenically banked in liquid nitrogen.

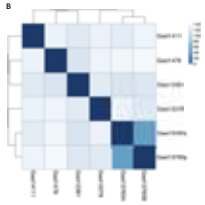
Drug sensitivity



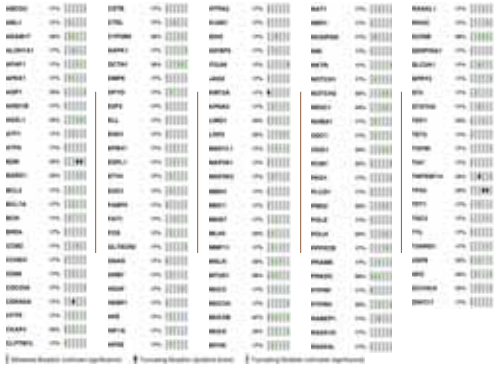
Waterfall plots showig the different treatment responses of our gastric cancer PDX towards the tested standard-of-care chemotherapeutics. Response rates are based on treated-to-control (T/C) values.
(> 50% T/C = resistance, 50-30% T/C = minor response, 30-5% T/C = moderate response, < 5% T/C = strong response)

Transcriptomic analyses





Unsupervised comparative genome-wide gene expression analysis based on STAR-generated RNASeq read counts normalized by variance stabilizing transformation (VST) using the *DESeq2* R-package.
(A) Principal component analysis (PCA) resulting in 3 groups possibly reflecting their histopathology. PCA done by R-package *factoextra*.
(B) Heatmap from the distance matrix with hierarchical clustering showing a similar grouping of models. Calculated by Euclidian distance and complete linkage; generated by *pheatmap* R-package.



OncoPrint of 174 expressed protein-changing mutations in 122 cancer-related genes detected by RNASeq variant calling including 5 truncating mutations in putative cancer driver genes. 2 of them are already drugable.
Samples are arranged as follows: **Gast1478 | Gast12278 | Gast12381 | Gast13765A | Gast13765B | Gast14111**
Graphic generated by *OncoPrinter* (cBioPortal.org, Gao et al. SciSignal 2013 & Cerami et al. CancerDiscovery 2012)

Conclusion

Our established set of gastric cancer PDX will serve as a valuable preclinical tool to investigate molecular mechanisms of drug sensitivity and resistance. The presence of certain deleterious (driver) mutations that are potentially druggable underlines Its potential to provide a source for translational research and for preclinical testing of new drug candidates.

Contact: **mibecker@epo-berlin.com**