1843

Intratumoral heterogeneity of renal cancer is related to differences in drug response and development of therapy resistance

M. Becker[1], B. Jandrig[2], S. Flechsig[1], R. Zeisig[1], D. Schindele[2], M. Schostak[2], C. Schmees[3], A. Wulf-Goldenberg[1], J. Hennenlotter[4], E. Schaeffeler[5], M. Schwab[5], A. Stenzl[4], J. Bedke[4], and J. Hoffmann[1]

[1] Experimental Pharmacology & Oncology Berlin-Buch GmbH, Berlin, Germany; [2]Department of Urology, University Medical Center Magdeburg, Germany; [3]NMI, University of Tübingen, Reutlingen, Germany; [4]Department of Urology, University Hospital Tübingen, Germany; [5]IKP Institut for Clinical Pharmacology, Stuttgart, Germany

Background and Aim

Patients with advanced renal cell cancer (RCC) have a poor prognosis not least because of resistance towards standard drugs. Recently, pronounced intratumoral heterogeneity (ITH) in RCC was shown. We were interested whether this ITH is a potential cause for treatment failure. We developed a large panel of patient-derived xenograft (PDX) models from RCC, including subsets of models from different regions of one individual patient tumor. The PDX models were evaluated for response to targeted standard therapeutics. To better understand correlations between inter- and intratumoral heterogeneity and treatment response, an explorative analysis of gene expression and panel sequencing data was performed.

Methods

Specimens from primary and metastatic RCCs were collected from consenting patients and transplanted into mice. Tumor engraftment was monitored for up to 4 months. Tumor sections were examined histopathologically to assess concordance between patient tumor and model and were stained for RCC specific markers (Pax2, Pax8, CD31, and RCC). Stable growing PDX were treated with the targeted compounds bevacizumab, sunitinib, sorafenib, and everolimus. Genome-wide gene expression was analyzed using Affymetrix® microarrays. In addition, sequence variations using the Illumina® NGS TSA cancer panel and *MET* and *TERT* gene copy numbers were analyzed in PDX models.

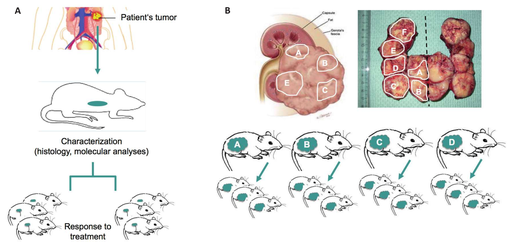
Results

A panel of 34 RCC PDX models was established from more than 200 patient samples. Among these, 13 models were derived from different tumor regions of three patients with advanced disease.

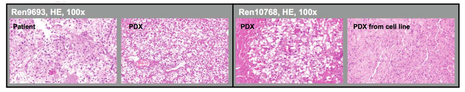
Original patient tumor and PDX showed a very similar and characteristic RCC histopathology. Inter- and intratumoral heterogeneity was preserved for several passages.

We treated all PDX with 4 standard targeted drugs and observed response rates comparable to results from clinical trials. One out of 8 regions obtained from one aggressive RCC (11175) clearly differentiated regarding its response to bevacizumab and sunitinib. Genomic analysis revealed that this region (11175D) has differences in global gene expression and mutational status. Besides a common *MET* mutation an additional variation in the *HRAS* gene was detected. Differential gene expression analysis of treatment responder/nonresponder groups resulted in weak sets of genes (low fold change) significant in moderated t-test but not significant after False Discovery Rate (FDR) adjustment, supposably because of a constrained assessment of true responder groups.

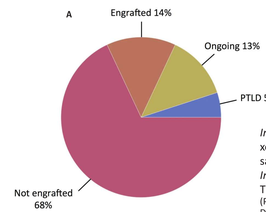
In the whole PDX set we found 34 sequence variations in 20 genes, e.g. *ATM*, *MET*, *TP53* and *VHL* and copy number variations in the *MET* locus (CNV data not shown).



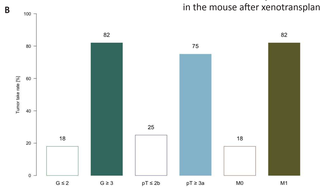
Generation of patient-derived xenograft models (PDX) in general routinely after surgery **(A)** and from different regions of one patient tumor (multi regions approach) **(B)**.

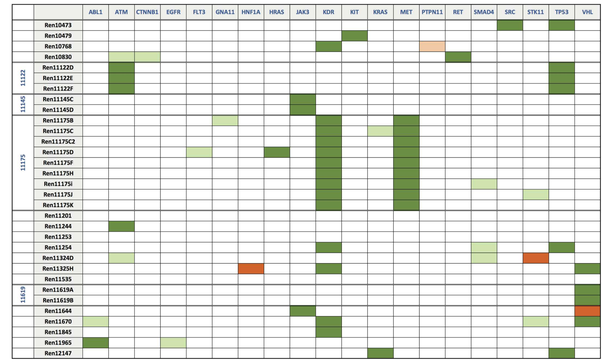


Histology of PDX generated directly from the patient tumor remains stable (left example Ren9693). Xenografts derived from primary RCC cell culture do not show the typical features of the original tumor (right example (Ren10786).

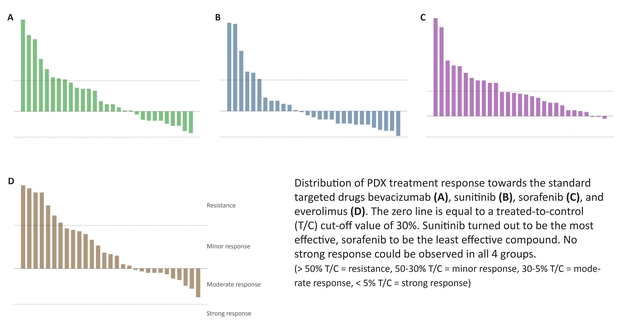


In vivo tumor take rates of 219 freshly xenotransplanted RCC patient tumor samples **(A)**.
In vivo tumor take rates stratified by TNM staging and grading **(B)**.
(PTLD = Post-Transplant Lymphoproliferative Disorder – A clinical phenomenon also occuring in the mouse after xenotransplantation)

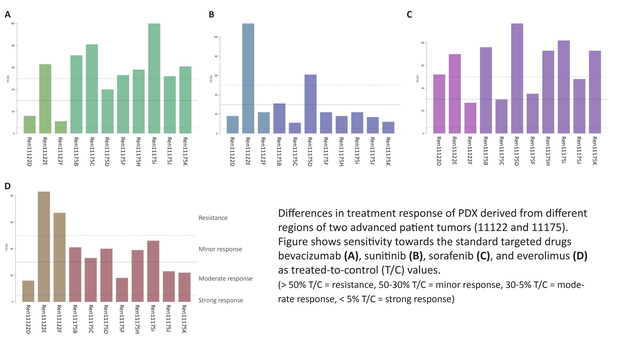




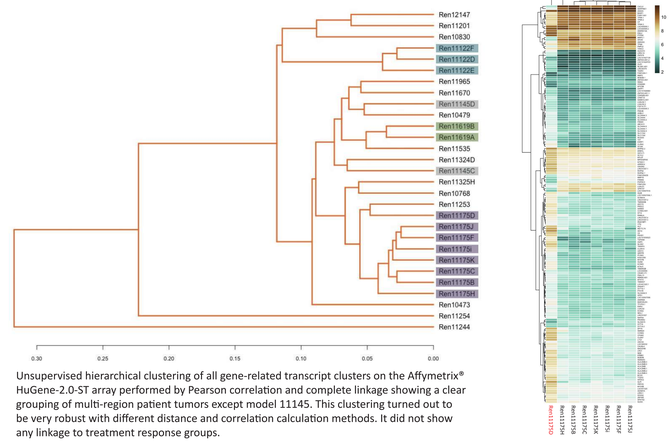
Genomic sequence variations of the RCC PDX models as detected by NGS using the Illumina® TrueSeq Amplicon Cancer panel (TSACP) performed on an Illumina MiSeq device. This panel covers 220 regions in 48 cancer-related genes. Single nucleotide variations (SNV's) are shown in green and insertions/deletions are shown in orange. Lightish = variations at low frequency (5-20%).



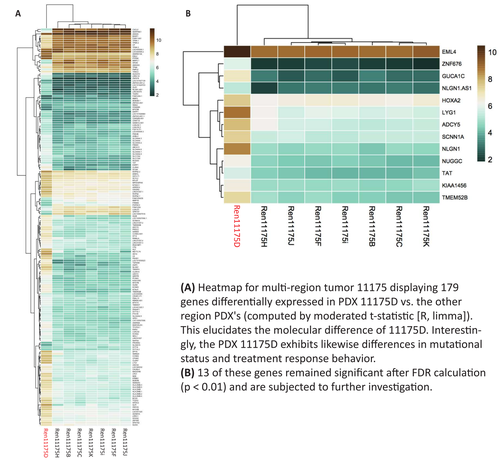
Distribution of PDX treatment response towards the standard targeted drugs bevacizumab **(A)**, sunitinib **(B)**, sorafenib **(C)**, and everolimus **(D)**. The zero line is equal to a treated-to-control (T/C) cut-off value of 30%. Sunitinib turned out to be the most effective, sorafenib to be the least effective compound. No strong response could be observed in all 4 groups.
(> 50% T/C = resistance, 50-30% T/C = minor response, 30-5% T/C = moderate response, < 5% T/C = strong response)



Differences in treatment response of PDX derived from different regions of two advanced patient tumors (11122 and 11175). Figure shows sensitivity towards the standard targeted drugs bevacizumab **(A)**, sunitinib **(B)**, sorafenib **(C)**, and everolimus **(D)** as 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)



Unsupervised hierarchical clustering of all gene-related transcript clusters on the Affymetrix® HuGene-2.0-ST array performed by Pearson correlation and complete linkage showing a clear grouping of multi-region patient tumors except model 11145. This clustering turned out to be very robust with different distance and correlation calculation methods. It did not show any linkage to treatment response groups.



(A) Heatmap for multi-region tumor 11175 displaying 179 genes differentially expressed in PDX 11175D vs. the other region PDX's (computed by moderated t-statistic [R, limma]). This elucidates the molecular difference of 11175D. Interestingly, the PDX 11175D exhibits likewise differences in mutational status and treatment response behavior.
(B) 13 of these genes remained significant after FDR calculation ($p < 0.01$) and are subjected to further investigation.

Conclusions

We have shown that PDX derived from distinct regions within one individual patient tumor can exhibit differences in genomic profile which possibly results in altered treatment response. This intratumoral molecular heterogeneity and its correlation to treatment response is subject of ongoing investigations to explain failure in renal cancer treatment.

The available panel of renal cancer PDX provides an excellent source for translational research and for preclinical testing of new drug candidates.

Contact: mibecker@epo-berlin.com

www.epo-berlin.com