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Kidney Cancer

Ex Vivo Drug Testing in Patient-derived Papillary Renal Cancer Cells Reveals EGFR and the BCL2 Family as Therapeutic Targets

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Abstract

Background: Immune checkpoint inhibitors and antiangiogenic agents are used for first-line treatment of advanced papillary renal cell carcinoma (pRCC) but pRCC response rates to these therapies are low.

Objective: To generate and characterise a functional ex vivo model to identify novel treatment options in advanced pRCC.

Design, setting, and participants: We established patient-derived cell cultures (PDCs) from seven pRCC samples from patients and characterised them via genomic analysis and drug profiling.

Outcome measurements and statistical analysis: Comprehensive molecular characterisation in terms of copy number analysis and whole-exome sequencing confirmed the concordance of pRCC PDCs with the original tumours. We evaluated their sensitivity to novel drugs by generating drug scores for each PDC.

Results and limitations: PDCs confirmed pRCC-specific copy number variations such as gains in chromosomes 7, 16, and 17. Whole-exome sequencing revealed that PDCs retained mutations in pRCC-specific driver genes. We performed drug screening with 526 novel and oncological compounds. Whereas exposure to conventional drugs showed low efficacy, the results highlighted EGFR and BCL2 family inhibition as the most effective targets in our pRCC PDCs.

Conclusions: High-throughput drug testing on newly established pRCC PDCs revealed that inhibition of EGFR and BCL2 family members could be a therapeutic strategy in pRCC.

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Patient summary: We used a new approach to generate patient-derived cells from a specific type of kidney cancer. We showed that these cells have the same genetic background as the original tumour and can be used as models to study novel treatment options for this type of kidney cancer.

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1. Introduction

Papillary renal cell carcinoma (pRCC) is the second most frequent renal cancer subtype after clear cell RCC (ccRCC). pRCC is a heterogeneous tumour entity with unpredictable patient outcomes [1,2]. Tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors have become the standard of care for patients with advanced RCC [3,4]. However, these treatment recommendations are largely orientated around on von Hippel-Lindau tumour suppressor–driven mechanisms in ccRCC [5] but are less effective in pRCC [4,6]. Drug development supported by underlying molecular mechanisms plays a critical role in identifying new personalised treatments. Depending on the genetic alteration, MET inhibitors have shown promising efficacy in MET-driven pRCC [7]. However, there is still a need for novel therapies in MET-independent pRCC. The lack of a targeted treatment for pRCC can be explained by the various molecular mechanisms involved in pRCC tumour biology. Moreover, new tools for renal tumours such as patient-derived cancer cell cultures (PDCs) that could help in exploring ex vivo drug responses are mainly focused on ccRCC biology [8,9]. In this study we generated PDCs to explore novel targeted treatments for pRCC. We investigated the molecular profile of these pRCC PDCs and compared their genomic landscape with that of the corresponding tumour tissue. We then performed a drug sensitivity and resistance testing assay with 526 compounds to identify shared and patient-specific ex vivo drug responses. Our results indicate that inhibition of EGFR and of BCL2 family members could be potential therapeutic strategies in pRCC.

2. Patients and methods

A detailed description of the patients and methods is available in the Supplementary material.

2.1. Ethic considerations

The Tissue Biobank at the University Hospital of Zurich (Switzerland) provided the tissue samples. All patients provided written consent for this study, which was authorised by the local ethics committee in Canton Zurich (BASEC 2019-01959). One normal tissue was processed as a part of the DEDUCER study, with written and dated ethics committee approval and patient consent (HUS/71/2017 and HUS/155/2021; Dnro 154/13/03/02/16 and HUS/850/2017).

3. Results

3.1. Mouse embryonic fibroblasts support the cellular growth of pRCC PDCs

We established a pRCC PDC model using mitotically inactivated CF6-Neo Mouse Embryonic Fibroblasts (MEFs) as a feeder layer. The pRCC PDCs were generated from a co-culture of MEFs seeded 24–48 h before addition of the cell suspension derived from digested tumour tissue (Fig. 1A). MEFs are mitotically inactivated but retain their potential to support cellular growth via the secretion of various hormones and growth factors to the culture media [10]. The optimal conditions for the PDCs were assessed using MEFs that were mitotically inactivated via either (1) treatment with mitomycin C (CF6-Neo) or (2) irradiation (CF1) for five different PDC/MEF ratios (1:1, 1:2, 2:1, 3:1, and 4:1; data not shown). Proliferation measurements showed that MEF-CF6 cells seeded at a ratio of 1:2 with MEF-conditioned media provided the most suitable conditions for supporting the growth of pRCC PDCs (Fig. 1B). Using this method, pRCC PDCs formed small nests on the feeder cells after 4 d of co-culture (Fig. 1C). After passaging the cells for 3–4 weeks, the feeder cells started to die, facilitating recovery of pure tumour cells derived from human pRCC tissue (Fig. 1A, D).

3.2. Molecular comparison between PDC and corresponding pRCC tissue

Genome-wide copy number variation (CNV) analysis of seven pRCC tissue samples and their matched PDCs showed overlapping CNV profiles, with several expected chromosomal aberrations (Fig. 2A). Recurrent cancer-specific alterations in pRCC in The Cancer Genome Atlas (TCGA) data sets [1], such as chromosome 7 and 17 gains, were the most frequent aberrations in our PDC models. Five PDCs had a gain in chromosome 16. Notably, three PDCs derived from paired grade 3 tumours (samples 1081, 300, and 764) had multiple chromosomal gains, while the other PDCs had only a few copy number alterations. Next, we compared somatic mutations shared by the primary tumour tissue samples and the pRCC PDCs using whole-exome sequencing (WES). Our analysis showed that five of the seven PDCs shared between 25% and 80% of the somatic mutations found in the parental tumour tissue, while two samples (195 and 764) were discrete (Fig. 2B). Samples 195_C and 764_C had CNV patterns identical to their paired tumour tissue, confirming that these two models were acceptable for the study. We then analysed relevant genes that were frequently mutated in our cohort after filtering for genes described in the TCGA and Catalogue of Somatic Mutations in Cancer (COSMIC) databases (Supplementary Fig. 1). The molecular profiles of the paired tumours and cell cultures resemble the complex molecular background of pRCC described in the TCGA data and also mirror the significant interpatient heterogeneity of mutated genes in pRCC (Fig. 2C). For example, mutations in the chromatin-

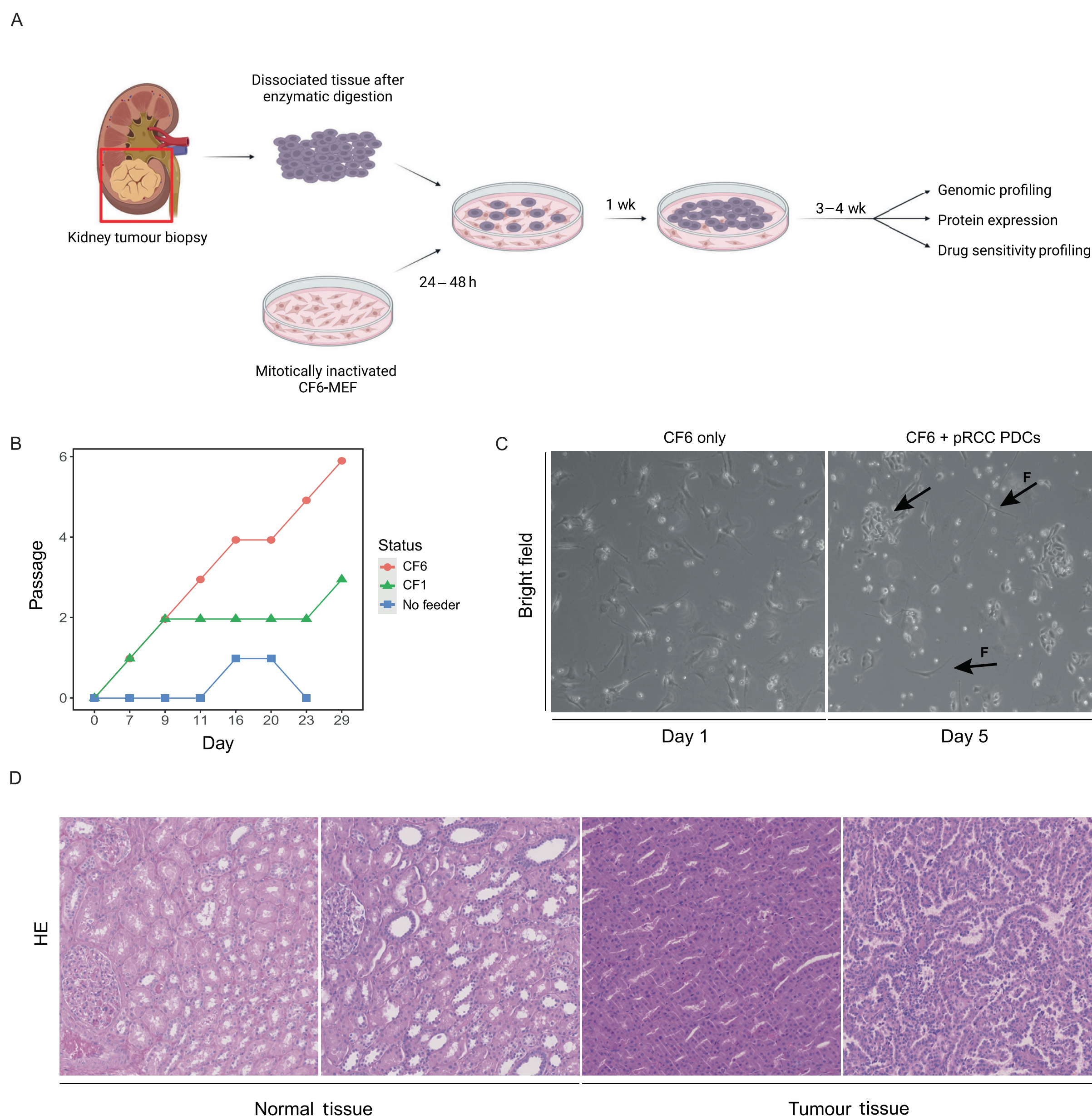

Fig. 1 – Establishment of papillary renal cell carcinoma (pRCC) patient-derived cell cultures (PDCs). (A) Schematic representation outlining the method used to generate PDCs from kidney biopsies using mitotically inactivated mouse embryonic fibroblasts (MEFs) as a feeder layer. Created with BioRender.com. (B) Growth curves and cell passages for pRCC PDCs in combination with MEFs mitotically inactivated via mitomycin C treatment (CF6) or irradiation (CF1), or without feeder cells (no feeder). (C) Bright-field images of CF6-MEF feeders alone (day 1) or in co-culture with pRCC PDCs after 5 d. Black arrows indicate feeder cells (F) or tumour cell nests (20× magnification). (D) Haematoxylin and eosin staining of two normal kidney tissues and of two tumour tissues with different morphology from which PDCs with different morphology were generated (10× magnification).

modifying gene *SETD2* were identified in one sample. Moreover, two samples showed mutations in *NFE2L2*, the key gene of the NRF2-ARE pathway.

Unsupervised clustering using gene variants also found within the TCGA and COSMIC databases revealed that matched pRCC tissues and PDCs clustered to the same branch for four of the seven samples (Fig. 2C, dendrogram). PDCs 193_C and 195_C did not cluster together with their paired tumours. Sample 195_C had the lowest mutational frequency among all the PDCs (only seven variants were identified), which may explain the discrepancy in the cluster analysis. However, CNV analysis confirmed the tumour background of the PDC model. Sample 193_C acquired several further variants during the culture passaging in addition to the mutations detected in the tumour, which changed the mutational landscape of 193_C. Nevertheless, 193_C and the paired tumour (193_T) shared chromosomal aberrations and mutations in two of the top mutated genes (*NFE2L2* and *PTPRS*), confirming that this culture represents an acceptable model for our study.

3.3. *Comprehensive drug testing shows distinct response profiles of PDCs from pRCC*

To define new potential therapeutic solutions for pRCC, we performed drug testing on six pRCC PDCs using a collection

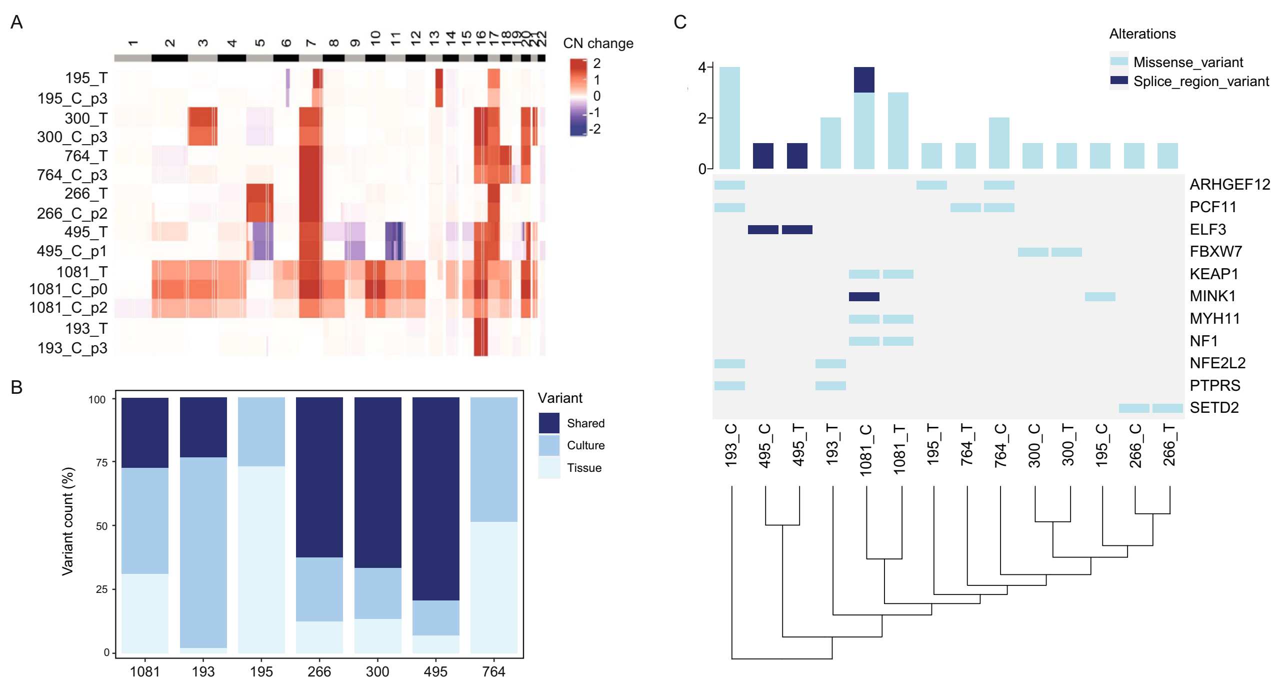

Fig. 2 – Genomic characterisation of papillary renal cell carcinoma (pRCC) patient-derived cell cultures (PDCs). (A) Genome-wide copy number (CN) variation analysis of parental pRCCs and matched PDCs. Red and blue bars indicate chromosomal gain or loss, respectively. (T = primary tumour; C = cell culture; p1–3 = passage numbers for cell cultures). (B) Whole-exome sequencing analysis of tumour and paired PDCs: bar plots showing the percentage of mutations shared between paired pRCC tumours and PDCs. (C) The most relevant genes in pRCC tissue (_T) and paired PDCs (_C) were determined by focusing on the set of genes implicated in The Cancer Genome Atlas study [1]. The dendrogram shows unsupervised clustering of pRCC tumours (_T) and paired PDCs (_C) for on all gene variants. The bars indicate the number of mutations found in each sample.

of 526 compounds over five concentrations [9] and measured cell viability after 72 h of treatment (Supplementary Fig. 2A, B). Sample 495_C was excluded because the corresponding cell culture did not grow to reach the 5 million cells needed for the drug screening. Therefore, the drug responses were analysed for six pRCC, one chromophobe RCC, and four normal kidney PDCs. We generated a selective drug sensitivity score (sDSS) for each compound using the normal kidney samples as controls (Supplementary Fig. 3). Tumour-specific sensitivity was defined as sDSS $\geq$5 and resistance as sDSS $\leq$−5.

Using these criteria, pRCC PDCs showed response to 52 compounds (out of 526) following the first screening (Supplementary Fig. 4A). Next, we performed drug screening with a minimum of two biological replicates to validate the sensitivity of the pRCC PDCs to the 52 active compounds. A significant sDSS was observed for 23 compounds: the PDCs were resistant to eight compounds (sDSS $\leq$−5), while 15 drugs were active in at least one PDC (Fig. 3A). Drug response curves and half-maximal inhibitory concentration (IC50) values highlighted the variability between the PDCs (Fig. 3B, C, Supplementary Fig. 5, and Supplementary Table 2). Enrichment analysis using drug-specific target sets showed that inhibition of EGFR is one of the primary mechanisms of action of the drugs in the PDCs (Fig. 3D, left panel). Afatinib, canertinib, and dacomitinib are irreversible inhibitors of EGFR and ERBB family members. Sample 300_C was the most responsive PDC to these compounds. In addition, we found that A-1331852 and A-1155463, two new chemical compounds, significantly reduced cell viability in five PDCs. These compounds target members of the BCL2 family such as BCL-XL. Accordingly, enrichment analysis showed that BMF (BCL2-modifying factor), BCL2L2 (BCL2-like protein 2) and HRK (Harakiri, BCL2-interacting protein) are targets of the 15 active compounds (Fig. 3D, right panel).

Finally, we investigated the effect of mTOR inhibitors and TKIs on our pRCC PDCs (Fig. 3E). These compounds are therapeutic agents currently used for pRCC; however, we found that these inhibitors had no effect (sDSS <5) on our pRCC PDCs. Interestingly, four samples (300, 764, 195, and 193) were resistant to everolimus and temsirolimus.

3.4. *Genomic alterations are associated with drug response profiles*

We evaluated the extent to which specific genetic features (eg, single nucleotide variants) affect drug responses at a pathway level. Of note, the ex vivo responses of PDCs can be influenced by genomic cancer alterations that make PDCs resistant or sensitive to compounds. We conducted a pattern association analysis between the somatic mutations identified from WES data and the sDSS results. To avoid spurious associations, we first mapped the gene mutations to the reactome pathways and generated a pathway-based mutational landscape. Next, the fully matched patterns for the drug responses and the pathway-related genetic mutations were identified (Fig. 4A). Three PDCs (764_C, 195_C, and 193_C) played a major role in pattern association analysis for two drugs, navitoclax and bortezomib. Response to navitoclax (sensitivity) and bortezomib (resistance) had the exact same patterns as the mutation aggregations in several

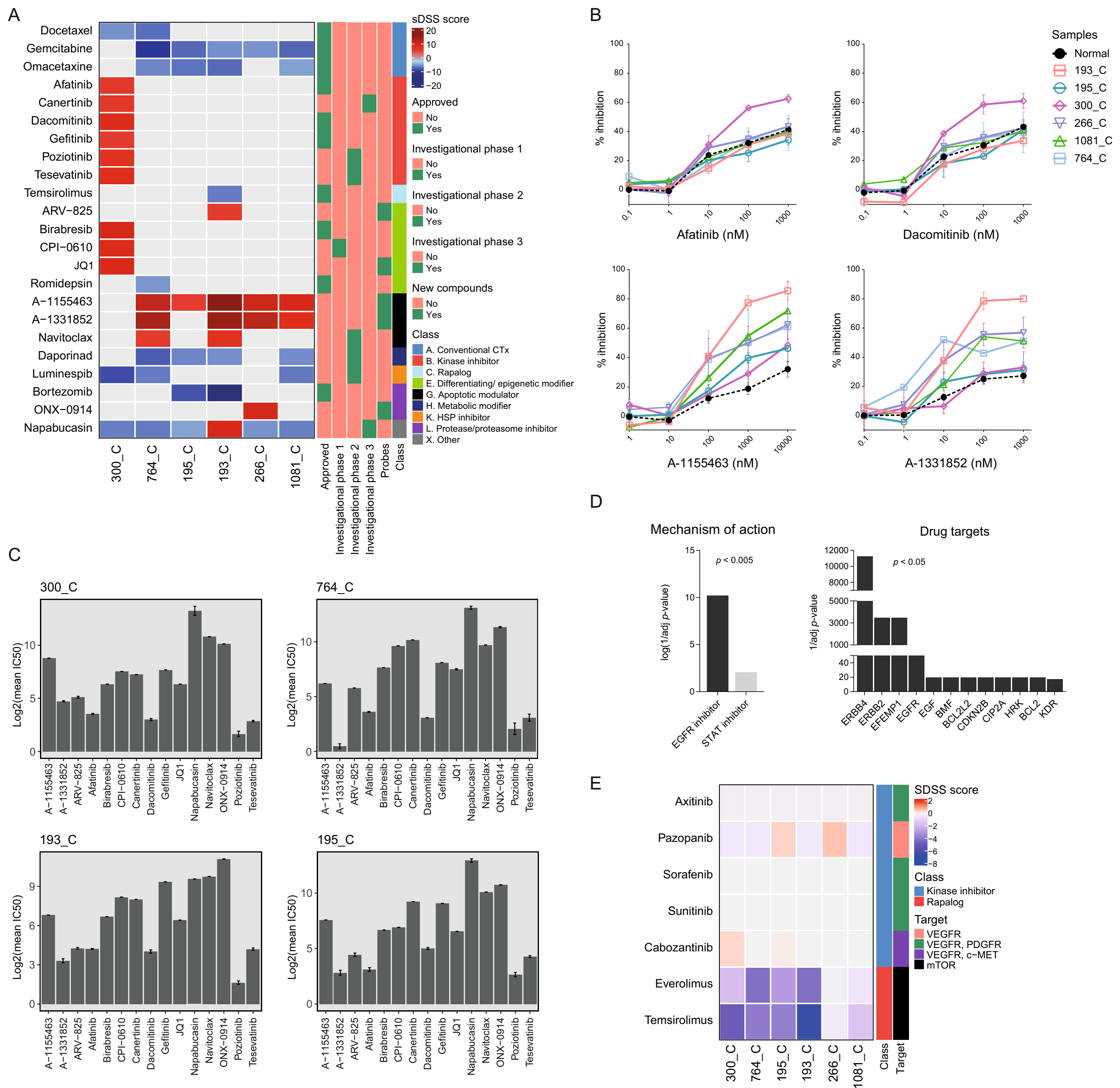

Fig. 3 – High-throughput drug screening on six papillary renal cell carcinoma (pRCC) patient-derived cell cultures (PDCs). (A) Heatmap of selective drug sensitivity scores (sDSS) in pRCC PDCs for 23 compounds with sDSS ≥5 (active drugs) versus ≤-5 (resistance). Blue and red lines denote drugs that PDCs were resistant to or sensitive to, respectively. For each compound, the class of action and investigational status are shown. (B) Dose-response curves for selected active drugs. The cell viability of six PDCs after 72 h was determined using five different concentrations for each drug. Data are presented as the mean ± standard error of the mean (SEM) for three independent replicates. (C) Half-maximal inhibitory concentration (IC50) values for selected drugs in four PDCs. The data are presented as the mean ± SEM for three independent replicates. (D) Mechanism of action and specific targets of selected drugs. DrugEnrichr analysis showed that inhibition of EGFR is the main mechanism of action of these compounds in our pRCC PDCs. (E) sDSS heatmap for seven standard-of-care drugs for pRCC. The drug class and targets are shown on the right. Blue and red lines denote the drugs that PDCs were resistant to or sensitive to, respectively.

pathways (Fig. 4B, C). Network analysis revealed how different mutated pathways can influence sensitivity to navitoclax or resistance to bortezomib (Fig. 4D). For example, the family of genes encoding the Rho GTPases, which play a role in various cancer types [11] and show differential expression in pRCCs [12], was also associated with sensitivity to navitoclax, a BCL2 inhibitor. Resistance to bortezomib in two samples (193_C and 195_C) correlated with mutations in two genes involved in rRNA regulation.

4. Discussion

There are only a few treatment options available for pRCC, none of which is very effective [13]. Our study represents an essential step towards functional precision medicine for pRCC: we successfully established pRCC PDCs, carried out functional drug testing of 526 compounds in PDCs, and identified associations between genetic variants and drug responses at the gene-pathway level. We established

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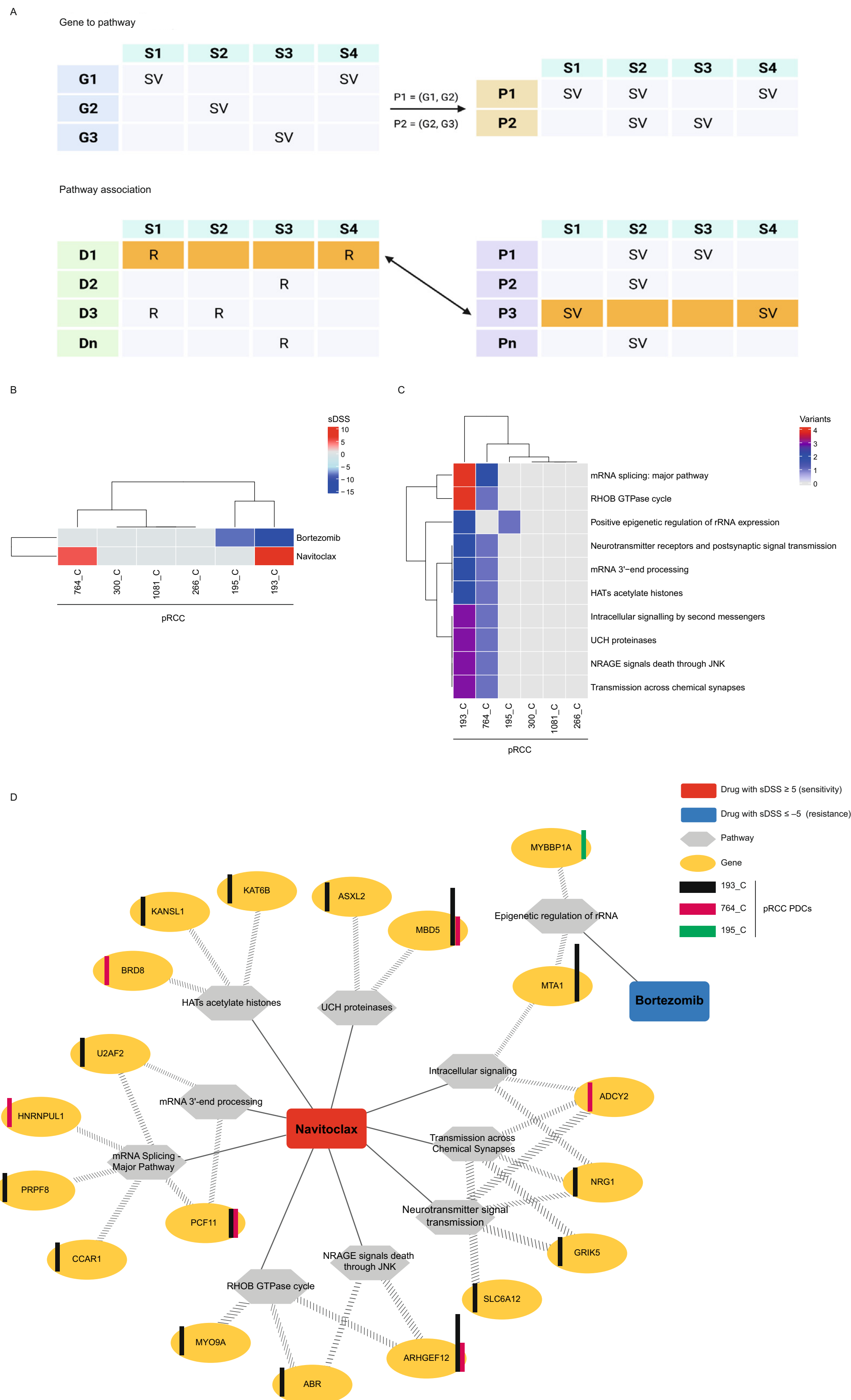

Fig. 4 – Pattern association for the mutational genetic landscape and drug response. (A) Drug-variant association analysis: genes with a mutation from each sample were mapped to the Reactome pathways. The matrix pathway/patient was compared with the exact drug-response scheme for each sample (G = gene; D = drug; P = pathway; S = sample; R = drug response; SV = single-nucleotide variant). (B) Heatmaps showing the drug responses of bortezomib (resistance) and navitoclax (sensitivity) in six papillary renal cell carcinoma (pRCC) patient-derived cell cultures. sDSS = selective drug sensitivity score. (C) Genetic variants within the Reactome pathways identified from the pattern association analysis. (D) Network showing the association of drug responses and potential causative pathways and genes mutated. The links between the pathways and drugs were obtained from the pattern association analysis (fully matched).

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PDCs from seven pRCC biopsies using MEFs as a feeder layer. The culture protocol supported successful growth of pRCC PDC models, as they all showed pRCC-specific copy number gains, mainly in chromosomes 7, 16, and 17. In addition, our PDCs recapitulated the most relevant mutated genes in pRCC and the mutations identified were shared with the corresponding primary tissue. Finally, we showed that the first mechanisms of action of the drugs on PDCs can be attributed to inhibition of EGFR and BCL2 family members.

The development of personalised treatment approaches has been slow for pRCC after the introduction of TKIs as standard treatment for RCC tumours [14]. MET-targeted therapies have shown promising results in MET-driven tumours [15]. For example, doretinib, a dual MET-VEGFR inhibitor, led to an overall response rate (ORR) of 50% versus 9% in pRCC with versus without germline *MET* mutations. In 2020, the SAVOIR trial investigated savolitinib, a highly selective MET inhibitor, versus sunitinib in patients with pRCC and *MET* alterations. The results indicated better efficacy and ORR (27% vs 7%) with savolitinib than with sunitinib [16].

MET overexpression has been observed in pRCC cells. Yang et al. [17] generated seven cell lines from pRCC samples from patients with MET mutations and chromosome 7 gain. These models showed response to two MET inhibitors, tepotinib and capmatinib, demonstrating the importance of preclinical models for therapeutic studies. Several immortalised RCC cell lines have been used to investigate mechanisms of drug resistance [18]. NCC-FH1 was proposed as a new FH-deficient pRCC cell line [19], but, according to the 2016 and 2022 World Health Organization RCC classification, such tumours are regarded as FH-deficient RCC, which has a different molecular background to pRCC. Identification of novel RCC entities has required a reinterpretation of currently available RCC cell lines and highlighted that PDCs represent a more adequate tool for recapitulating tumour complexity in vitro [20]. Our PDCs identified gains for chromosomes 7 and 17 as recurrent pRCC alterations, but no mutations in *MET*, confirming the rare occurrence of *MET* mutations in sporadic pRCC [21].

Our drug screening identified a role for EGFR inhibition in pRCC. Although *EGFR* mutations were found only in 0.4% of pRCCs according to TCGA data on cBioPortal, we have shown that EGFR upregulation is frequent in pRCC [22]. It has also been demonstrated that EGFR hyperactivation is a poor prognostic marker in ccRCC [23] and non–small-cell lung cancer [24]. Moreover, erlotinib, an EGFR inhibitor used in lung adenocarcinoma, showed promising activity in patients with hereditary leiomyomatosis and RCC [25].

Pal et al. [26] showed that a group of advanced pRCCs was characterised by concurrent mutations in *MET* and *EGFR*. Combinations of erlotinib and MET inhibitors have been investigated in several solid tumours [15], suggesting potential use of this approach in pRCC. Our results indicate that treatment options for pRCC may be broadened using the MET-EGFR axis as a therapeutic target.

Our studies highlighted that two new chemical compounds, A-1155463 and A-1331852, elicited a response in five pRCC PDCs, suggesting targeted specificity for pRCC. These molecules are BCL-XL-specific inhibitors and belong to the apoptotic modulator family. Studies on colorectal cancer cell lines treated with these compounds demonstrated strong inhibition of cellular growth [27]. Moreover, RCC cell lines with high BCL2 expression showed higher sensitivity to treatment with BCL2 inhibitors in combination with cisplatin, suggesting that this mechanism should also be further explored in pRCC [28].

Recent studies have shown that treatment with EGFR inhibitors in combination with Navitoclax can cause a significant tumour reduction in a patient-derived xenograft model of EGFR-expressing breast cancer, suggesting the potential efficacy of navitoclax in EGFR positive tumours [29]. Efficacy of Navitoclax, an antagonist of the antiapoptotic BCL-XL, was observed in two of our pRCC PDCs, suggesting that combined use of EGFR inhibitors and apoptosis modulators may represent a targeted approach in pRCC. Future investigations will help in understanding the potential synergistic effect of these compounds in pRCC.

The establishment of pRCC models will shift therapy options to better-individualised treatments. In our work, pRCC PDCs were established on the basis of a method described by Saeed et al. [9], who used irradiated mouse fibroblasts (3T3 cells) as a feeder layer in ccRCC. We found that MEFs mitotically inactivated via mitomycin C treatment can sustain cellular growth by secreting several important growth factors and adhesion molecules into the medium and provide a cellular matrix for cells [10].

Maintenance of tumour cell cultures from cancer tissue sample has several challenges. Contamination and overgrowth of tumour cells by normal kidney cells or fibroblasts have been reported in many studies and observed in our daily experience [30]. These limitations strictly justify the need for accurate molecular validation of PDCs in comparison to the parental tumour. Here, the pRCC specificity of our PDCs was demonstrated via CNV and gene mutation analyses of the PDCs and their paired tumours. Although the sample size may represent a possible limitation to this study, we showed that our pRCC PDCs can recapitulate tumour heterogeneity. pRCC PDCs provide a valuable resource for reliable drug discovery and, in combination with the genetic landscape of pRCC, can improve personalised treatment options for patients with pRCC.

5. Conclusions

In conclusion, we propose that pRCC PDCs offer a functional tool to represent the complexity of pRCC heterogeneity and to investigate novel drug compounds that could efficiently and specifically target pRCC. Our findings point to alternative possibilities for the clinical management and diagnosis of patients with advanced pRCC.

Author contributions: Holger Moch had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Moch, Schraml, Angori, Pietiäinen.

Acquisition of data: Angori, Mühlbauer.

Analysis and interpretation of data: Angori.

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Supervision: Moch, Schraml, Pietiäinen.
Other (computational analysis and data visualization): Banaei-Esfahani, Kahraman, Karakulak.
Other (development of the methodology and the design of the workflow): Angori, Mühlbauer, Bolck.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euf.2023.03.005.

References

[1] The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of papillary renal-cell carcinoma. N Engl J Med 2016;374:135–45.

[2] Moch H, Amin MB, Berney DM, et al. The 2022 World Health Organization classification of tumours of the urinary system and male genital organs—part A: renal, penile, and testicular tumours. Eur Urol 2022;82:458–68.

[3] Powles T, Albiges L, Bex A, et al. ESMO Clinical Practice Guideline update on the use of immunotherapy in early stage and advanced renal cell carcinoma. Ann Oncol 2021;32:1511–9. https://doi.org/10.1016/j.annonc.2021.09.014.

[4] Pal SK, Tangen C, Thompson Jr IM, et al. A comparison of sunitinib with cabozantinib, crizotinib, and savolitinib for treatment of advanced papillary renal cell carcinoma: a randomised, open-label, phase 2 trial. Lancet 2021;397:695–703.

[5] The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 2013;499:43–9.

[6] Brodziak A, Sobczuk P, Bartnik E, et al. Drug resistance in papillary RCC: from putative mechanisms to clinical practicalities. Nat Rev Urol 2019;16:655–73. https://doi.org/10.1038/s41585-019-0233-z.

[7] Choueiri TK, Heng DYC, Lee JL, et al. Efficacy of savolitinib vs sunitinib in patients with MET-driven papillary renal cell carcinoma: the SAVOIR phase 3 randomized clinical trial. JAMA Oncol 2020;6:1247–55.

[8] Kazama A, Anraku T, Kuroki H, et al. Development of patient-derived tumor organoids and a drug testing model for renal cell carcinoma. Oncol Rep 2021;46:226.

[9] Saeed K, Ojamies P, Pellinen T, et al. Clonal heterogeneity influences drug responsiveness in renal cancer assessed by ex vivo drug testing of multiple patient-derived cancer cells. Int J Cancer 2019;144:1356–66.

[10] Firas J, Liu X, Nefzger CM, Polo JM. GM-CSF and MEF-conditioned media support feeder-free reprogramming of mouse granulocytes to iPS cells. Differentiation 2014;87:193–9.

[11] Haga RB, Ridley AJ. Rho GTPases: regulation and roles in cancer cell biology. Small GTPases 2016;7:207–21.

[12] Zhang K, Lee HM, Wei GH, Manninen A. Meta-analysis of gene expression and integrin-associated signaling pathways in papillary renal cell carcinoma subtypes. Oncotarget 2016;7:84178–89.

[13] Fenner A. Diving into the genome of papillary RCC unearths therapeutic pearls. Nat Rev Urol 2017;14:453.

[14] Angori S, Lobo J, Moch H. Papillary renal cell carcinoma: current and controversial issues. Curr Opin Urol 2022;32:344–51.

[15] Rhoades Smith KE, Bilen MA. A review of papillary renal cell carcinoma and MET inhibitors. Kidney Cancer 2019;3:151–61.

[16] Yamana K, Ohashi R, Tomita Y. Contemporary drug therapy for renal cell carcinoma—evidence accumulation and histological implications in treatment strategy. Biomedicines 2022;10:2840.

[17] Yang Y, Ricketts CJ, Vocke CD, et al. Characterization of genetically defined sporadic and hereditary type 1 papillary renal cell carcinoma cell lines. Genes Chromosomes Cancer 2021;60:434–46.

[18] Brodaczewska KK, Szczylik C, Fiedorowicz M, Porta C, Czarnecka AM. Choosing the right cell line for renal cell cancer research. Mol Cancer 2016;15:83.

[19] Perrier-Trudova V, Huimin BW, Kongpetch S, et al. Fumarate hydratase-deficient cell line NCCFH1 as a new in vitro model of hereditary papillary renal cell carcinoma type 2. Anticancer Res 2015;35:6639–53.

[20] Li Z, Xu H, Yu L, et al. Patient-derived renal cell carcinoma organoids for personalized cancer therapy. Clin Transl Med 2022;12:e970.

[21] Schmidt L, Junker K, Nakaigawa N, et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene 1999;18:2343–50.

[22] Moch H, Sauter G, Gasser TC, et al. EGF-r gene copy number changes in renal cell carcinoma detected by fluorescence in situ hybridization. J Pathol 1998;184:424–9.

[23] Đorđević G, Matušan Ilijaš K, Hadžisejdić I, Maričić A, Grahovac B, Jonjić N. EGFR protein overexpression correlates with chromosome 7 polysomy and poor prognostic parameters in clear cell renal cell carcinoma. J Biomed Sci 2012;19:40.

[24] Selvaggi G, Novello S, Torri V, et al. Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol 2004;15:28–32.

[25] Wyvekens N, Valtcheva N, Mischo A, et al. Novel morphological and genetic features of fumarate hydratase deficient renal cell carcinoma in HLRCC syndrome patients with a tailored therapeutic approach. Genes Chromosomes Cancer 2020;59:611–9.

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[26] Pal SK, Ali SM, Yakirevich E, et al. Characterization of clinical cases of advanced papillary renal cell carcinoma via comprehensive genomic profiling. Eur Urol 2018;73:71–8.
[27] Lim B, Greer Y, Lipkowitz S, Takebe N. Novel apoptosis-inducing agents for the treatment of cancer, a new arsenal in the toolbox. Cancers 2019;11:1087.
[28] Kausch I, Jiang H, Thode B, Doehn C, Krüger S, Jocham D. Inhibition of bcl-2 enhances the efficacy of chemotherapy in renal cell carcinoma. Eur Urol 2005;47:703–9.
[29] Zoeller JJ, Vagodny A, Daniels VW, et al. Navitoclax enhances the effectiveness of EGFR-targeted antibody-drug conjugates in PDX models of EGFR-expressing triple-negative breast cancer. Breast Cancer Res 2020;22:132.
[30] Karthaus WR, Iaquinta PJ, Drost J, et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. Cell 2014;159:163–75.

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