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Tumor Xenografts of Human Clear Cell Renal Cell Carcinoma But Not Corresponding Cell Lines Recapitulate Clinical Response to Sunitinib: Feasibility of Using Biopsy Samples

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Abstract

Background—Parallel development of preclinical models that recapitulate treatment response observed in patients is central to the advancement of personalized medicine.

Objective—To evaluate the use of biopsy specimens to develop patient-derived xenografts and the use of corresponding cell lines from renal cell carcinoma (RCC) tumors for the assessment of histopathology, genomics, and treatment response.

Design, setting, and participants—A total of 74 tumor specimens from 66 patients with RCC were implanted into immunocompromised *NOD-SCID IL2R γ ^{-/-}* mice. Four cell lines generated from patients' specimens with clear cell pathology were used for comparative studies.

Outcome measurements and statistical analysis—Preclinical models were established and assessed. Engraftment rates were analyzed using chi-square testing. Analysis of variance (two-way analysis of variance) was conducted to assess tumor growth.

Results and limitations—Overall, 33 RCC mouse xenograft models were generated with an overall engraftment rate of 45% (33 of 74). Tumor biopsies engrafted comparably with surgically resected tumors (58% vs 41%; $p = 0.3$). Xenograft tumors and their original tumors showed high fidelity in regard to histology, mutation status, copy number change, and targeted therapy response. Engraftment rates from metastatic tumors were higher but not more significant than primary tumors (54% vs 34%; $p = 0.091$). Our engraftment rate using metastases or biopsies was comparable with recent reports using resected primary tumors. In stark contrast to corresponding cell lines, all tested xenografts recapitulated patients' clinical response to sunitinib.

Conclusions—Patient-derived xenograft models can be effectively established from tumor biopsies. Preclinical xenograft models but not matched cell lines reflected clinical responses to sunitinib.

Patient summary—Matched patient-derived clear cell renal cell carcinoma xenografts and cell lines from responsive and refractory patients treated with sunitinib were established and evaluated for pharmacologic response to anti-vascular endothelial growth factor treatment. Both models accurately reflected the genetic characteristics of original tumors, but only xenografts recapitulated drug responses observed in patients. These models could serve as a powerful platform for precision medicine.

Keywords

Patient-derived xenografts; Precision medicine; Preclinical studies; Renal cell carcinoma; Biopsy; Sunitinib; Cancer cell lines

1. Introduction

The most lethal genitourinary cancer is renal cell carcinoma (RCC), with >60 000 cases diagnosed and >14 000 deaths in the United States in 2015 [1]. For patients who have metastatic renal cell carcinoma (mRCC), the current standard of care is systemic treatment with targeted agents including inhibitors of vascular endothelial growth factor receptor pathways [2,3]. The response rate to these agents varies from 5% to 40% and is largely unpredictable [4]. With the advent of next-generation sequencing, several large collaborative studies have contributed to a better understanding of the genomic underpinnings of RCC [5–11]. In addition, the wide variety of new therapeutic options available for this disease has led to a need for improved methods in selecting the most efficacious drug for each patient beyond patient demographics [12–14].

Treatment for patients with cancer has recently shifted to a more personalized or so-called precision medicine approach [15,16], and mutations of clear cell renal cell carcinoma (ccRCC) could be predictive of treatment response to targeted therapy [17]. Central to this approach is the integration of novel molecular and targeted discoveries with clinical management and treatment responses. Commercially available RCC models largely rely on cell cultures that often fail to reflect reliably the pathophysiology and treatment responses achieved in patients. One method for overcoming this limitation is the development of patient-derived xenograft models, previously described [18–20]. RCC xenograft models have classically relied on tissue obtained by surgical resection, limiting the possibility of creating models of tumors that are not surgically removed.

We report the development of a cohort of stable xenograft models for RCC that incorporate the use of biopsy tissue and accurately reflect the genetic characteristics and the response to sunitinib treatment of the original tumors.

2. Materials and methods

On approval from our institutional review board, we identified patients with a clinical or pathologic diagnosis of RCC who were seen in either our urologic or medical oncology clinics. Patients were nonconsecutive and chosen for xenograft model creation on the basis of their preoperative or procedural imaging. Patients with multiple, bilateral, or recurrent tumors, suspicion of vascular or organ invasion, lymphadenopathy, rare histology, and metastatic disease were approached for xenograft creation. Most of the patients had advanced or metastatic disease at the time when they consented to participate in the study. This included patients with known or suspected hereditary RCC syndromes. Patients were excluded from the study if there was suspicion for non-RCC malignancy.

We generally passaged each tumor two to three times in vivo before collecting the xenograft tumors for histology analysis, live tissue banking, and frozen tissue stock. Live tissues banked in fetal bovine serum (FBS) with 10% dimethyl sulfoxide in liquid nitrogen were thawed out and used to generate xenograft tumors successfully. Clinical information was recorded from the medical reports provided by clinicians involved in the patients' care, and

objective radiologic tumor assessment were made according to Response Evaluation Criteria in Solid Tumors v.1.1.

2.1. Animals

All animal work was performed in accordance with institutional guidelines and institutional animal care and use committee (IACUC) approval. We used 5- to 6-wk-old male immunocompromised *NOD-SCID IL2R γ ^{-/-}* (NSG) mice (Jackson Laboratory) for the study.

2.2. Specimen collection

From June 2011 to February 2016, we collected tumor samples from a total of 66 patients. Samples of primary tumors or metastatic tumors were taken at the time of surgical or interventional procedures and were examined by institutional pathologists. Biopsy specimens were obtained from core needle biopsies. In one case, the specimen was obtained from aspiration of malignant ascites fluid and classified as a biopsy for the purpose of our analysis. All materials were kept on ice and stored in Roswell Park Memorial Institute (RPMI) medium or phosphate-buffered saline (PBS) until time of processing.

2.3. Sample processing

Tumor samples were processed within 16 h of tissue collection. Three methods were used to generate xenograft models in mice: surgically resected tissue implantation, biopsy implantation, and primary tumor cell injection. For surgically resected tissue implantation, mouse was anesthetized by inhalation with an isoflurane vaporizer. Mouse was placed face down, fur was removed, and the area was sterilized with three sets of alternating scrubs of Betadine and an alcohol pad. A 5-mm skin incision was made over the midlumbar spine, and the skin was bluntly dissected. Tumor samples were cut into 8- to 27-mm³ fragments and implanted into one or two sides of the flank subcutaneously. The incision was then closed with wound clips. For biopsy samples, core biopsy tissue was maintained in RPMI medium on ice before implantation. The mouse was prepared as previously described. One core biopsy tissue was implanted on one side of the flank subcutaneously. For primary tumor cell injection, tumor tissue was dissociated, cultured, and 1–6 million cells resuspended in PBS: Matrigel (Corning, NY, USA) (1:1) was injected into the flank of the mice subcutaneously. For all xenograft assays, tumor volume was measured biweekly and calculated using the formula $0.5 \times L \text{ (length)} \times W \text{ (width)} \times W \text{ (width)}$.

2.4. Dissociation and culture of primary kidney cancer cells

Tumor tissues were minced into small pieces and digested in dissociation buffer containing 225 U/ml Collagenase type III at 37°C for 3 h. Samples were then washed with advanced Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12; Life Technologies, Carlsbad, CA, USA), treated with Red Blood Cell Lysis Buffer (Sigma, St. Louis, MO, USA) for 2 min, washed again, filtered through 70- μ m cell strainers, and cultured in F10 medium (advanced DMEM/F12) with 10% FBS, 1 $\times$ L-glutamine, 1 $\times$ sodium pyruvate, 1 $\times$ nonessential amino acid, and 1 $\times$ penicillin/streptomycin).

2.5. Histologic examination

Tissue was processed, and hematoxylin and eosin staining was performed according to standard protocols. For immunohistochemistry staining, carbonic anhydrase IX (CAIX) antibody (NB100-417; Novus, Littleton, CO, USA) was used, and slides were read by a board-certified genitourinary pathologist (Y.B.C.).

2.6. Molecular characterization

DNA from both the patient's original tumor and the corresponding xenograft tumor was obtained from several sources on the basis of availability, including fresh tissue, dissociated cells, and formalin-fixed paraffin-embedded tissue. Sequencing analysis was performed using Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) [21]. This assay uses custom oligonucleotides designed to capture all protein-coding exons and select introns of commonly altered oncogenes and tumor suppressor genes (Supplement 1). This panel currently includes 410 genes. However, some tissue samples included in this study underwent sequencing with earlier gene panels that contained 341 and 230 genes. In the comparative analysis of matched patient tumors and xenograft tumors, genes not present in both panels (eg, present in the 410 panel but not in the 341 panel) were excluded.

2.7. Cell Titer-Glo luminescent cell viability assay

To test cell viability upon sunitinib treatment, cancer cells were plated at 3000 per well of 96-well plates and incubated overnight at 37°C with 5% carbon dioxide. On the following day, sunitinib was added to the wells; cultures were incubated for an additional 72 h. Cell viability was determined using the Promega Cell Titer-Glo luminescent cell viability kit (Madison, WI, USA) according to the manufacturer's instructions. The half maximal effective concentration (EC₅₀) value was calculated by nonlinear regression analysis using GraphPad Prism software v.5.02 (La Jolla, CA, USA).

2.8. In vivo response to therapy

In vivo treatment was started when tumor volumes reached 300–400 mm³. Mice were then randomly assigned into vehicle and treatment groups (6–12 tumors in each arm). The treatment group was given 60 mg/kg sunitinib in PBS by oral gavage daily. Following guidelines set forth by the IACUC, mice were killed when tumors length reached 2 cm. Tumor volume was measured and growth curves were generated using GraphPad Prism software.

2.9. Statistical analysis

A xenograft was deemed successful when the tumor volume reached 1.5 cm³ or the maximum tumor diameter reached 2 cm and RCC was confirmed by histopathology of the grafted tumors. Clinical variables of patients and differences in engraftment rates were analyzed using chi-square tests for categorical variables. Treatment effects on tumor growth were evaluated by two-way analysis of variance. Testing was done using GraphPad Prism software.

3. Results

3.1. Establishment of tumor xenografts

We generated 33 successful xenograft models among 74 specimens (45%) from 66 patients (Table 1). Tumor tissue derived from metastases led to a higher rate of success in generating xenograft models compared with tissue derived from primary tumors, but the difference was not statistically significant (54% vs 34%; $p = 0.091$). A higher fraction of biopsies led to successful xenografts than those from surgically resected tissue, but this was not statistically significant (58% vs 42%; $p = 0.30$) (Table 1).

The following studies focused on evaluating matched preclinical xenograft and cell line models derived from four clinically well-annotated ccRCC patients who received sunitinib as the first-line targeted therapy and exhibited either prolonged response (JHRCC3 from primary tumor; JHRCC62 from metastatic tumor) or immediate resistance (JHRCC12 from metastatic tumor; JHRCC228 from primary tumor).

3.2. Comparable histology

To confirm that our xenograft tumors accurately represented the original patient tumors, we performed comparative histologic and morphologic examination of the select patient's original tumor with the corresponding xenograft tumor (Fig. 1). Hematoxylin and eosin stains along with the immunohistochemical staining of CAIX were used in all xenograft tumors. Available cases for examination showed congruence among morphologic features between the original tumor and the corresponding xenograft tumor (Fig. 1 and Supplementary Fig. 1).

3.3. Retained molecular characteristics

Molecular characterization of four patients' original tumors and the corresponding xenograft models was performed using the MSK-IMPACT platform (Fig. 2). These four cases were likely sporadic, and patients had no known family history of RCC. Results in these cases demonstrated a high fidelity of somatic mutations in matched tumor samples, 24 of 27 genes (88.8%). We also detected comparable chromosomal copy number alterations between matched tumor samples (Fig. 2). One noted difference is an *MAGI2* deletion that is present in the primary tumor but absent in the corresponding xenograft tumor.

3.4. Selection of patient tumors with known primary clinical response or resistance to first-line sunitinib therapy

Based on commercially available ccRCC cell lines, inconsistent mechanistic models have been proposed regarding whether antiangiogenic drugs such as sunitinib primarily target tumor blood vessels (ie, tumor microenvironment and/or cancer cells) directly [22,23]. Among our xenograft cohort, we selected four patients with mRCC who were treated with first-line tyrosine kinase inhibitors (TKIs) and exhibited either primary response or primary resistance for further drug treatment studies. Two patients, JHRCC3 and JHRCC62, were radiographically responsive to first-line TKI therapy with treatment lengths of 1010 and 1297 d, respectively (Fig. 3). The other two patients, JHRCC12 and JHRCC228, were found

to be unresponsive, showing progression of disease to first-line TKI therapy with treatment durations of 31 and 55 d, respectively (Fig. 3).

3.5. Correlation of patient's sunitinib treatment response with corresponding tumor-derived xenograft and cell line

We performed in vitro sunitinib treatment studies on these four JHRCC cell lines (Fig. 4a) and evaluated sunitinib therapeutic responses in matched xenografts (Fig. 4b). We did not detect discernible differences in EC_{50} for cell viability with sunitinib treatment (Fig. 4a), whereas xenograft models demonstrated an analogous response to sunitinib therapy of all four patients (Fig. 4b).

4. Discussion

Previously described models have largely relied on primary tumors from surgically resected tissue to create patient-derived RCC xenografts [18,19,24]. The improved engraftment rate of metastatic tissue in RCC was previously reported by Sivanand et al on the basis of five cases [19]. Our xenograft models reproduce this finding with a larger cohort (21 successful xenograft models from metastatic tissue) and also demonstrate the feasibility of biopsy specimens as a minimally invasive approach to develop RCC xenograft models.

In our study, 10 of 15 biopsy engraftments grew to tumor in vivo with a successful rate (58%), which is higher than surgical tissue engraftments (42%) (Table 1). Reasons for this difference could relate to several confounding variables including selection of those with more disseminated or aggressive disease for biopsy versus surgical resection. Nonetheless these results demonstrate the viability and stability of using biopsy tissue to generate xenograft models. This may be an ideal approach for patients who are never considered for surgical resection or those who only have a planned biopsy prior to systemic therapy.

The ability to generate accurate models from metastatic tissue is central to the pursuit of a precision medicine approach in patient care. Karam et al [18] established four ccRCC xenografts and demonstrated treatment response with sunitinib and everolimus. Similar findings of response to various targeted agents using xenograft models were reported by Sivanand et al [19] and Thong et al [24]. However, none of these published models reported associated clinical responses of the original patients to targeted therapeutic agents. To the best of our knowledge, this study is the first to evaluate a direct correlation between a patient's clinical response to TKI therapy and the response of the corresponding xenograft model. More importantly, in vitro studies using the same tumor-derived cells were unable to provide discernible results or imitate the patients' clinical response.

It has been well documented that both primary and metastatic tumors in RCC harbor private mutations and regional genomic heterogeneity [25,26]. These metastatic tumors may include important driver mutations and possibly mutations that could predict response or resistance to targeted therapies. Given these recent findings we have now furthered our development of xenograft models mainly using specimens from metastatic lesions.

Because ccRCC is the most prevalent and best studied kidney cancer with unique genetic and metabolic changes [11,27–29], most of the RCC models published earlier focused on ccRCC. However, there is an urgent need to develop preclinical models for non-clear cell RCC (nccRCC) [29]. In our report, we successfully generated 11 patient-derived xenograft models for nccRCCs including papillary, translocation, medullary, and unclassified RCCs (Table 1). The use of information garnered from these models for clinical management appears to be on the horizon, although factors including cost and resources may limit its implementation. The use of cell lines derived from these infrequent tumor types, regardless of their ability to generate xenografts, can serve as useful platforms for biomarker discovery, a novel drug efficacy test, and a drug resistance mechanism study.

Limitations of our study include the selection bias of patients that were chosen for xenograft creations and by those who were referred to our tertiary care center. Also, most xenografts are derived from predominant genetic clones present in the tissues used for generation. Hence such models are unlikely to encompass all clones in a given individual patient. The consequence testing and experimentation with these models may not be representative of all the clinically relevant clones in a patient with metastatic disease. Lastly, due to limited sample size, data presented here warrant further validation with additional large cohorts in the future.

5. Conclusions

We established a cohort of xenograft models that recapitulate human RCC tumors and demonstrate analogous treatment response to sunitinib therapy. The use of biopsy tissue from metastatic lesions produced accurate models that may provide a pragmatic minimal invasive approach in developing real-time xenograft models for personalized drug testing and precision medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Comparative histologic examination between original tumor and patient-derived xenograft tumors. Representative hematoxylin and eosin-stained sections and carbonic anhydrase IX stain of patients' tumors and corresponding xenograft tumors. All cases are of clear cell histology, and all tissues were collected prior to the initiation of systemic therapy. Scale bar = 100 μ m.

CAIX = carbonic anhydrase IX; H&E = hematoxylin and eosin.

Fig. 2.

Mutation profiles and copy number plots for four cases with matched patient and xenograft tumors. Median coverage of all tumor samples was approximately 402×. Mutation profiles demonstrate high fidelity between patients and corresponding xenograft tumors with only three mutations (*AKT1* in JHRCC3, *PIK3CG* in JHRCC62, and *LATS1* in JHRCC228) not present in the xenograft sample. Copy number plots were calculated according to the presence and depth of chromosomal and genetic aberrations using patient blood for normal control. Respective tumor:normal ratios (y-axis) varied depending on the magnitude of these

aberrations. Enrichment for genetic aberrations in the xenograft models was seen in several instances (eg, *STK11* in JHRCC12 and *CDKN2A/CDKN2B* in JHRCC62). In one instance (JHRCC12), we noted the absence of a genetic deletion in the xenograft tumor that was present in the patient tumor (*MAGI2*).

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Fig. 3.

Computed tomography imaging of targeted lesions in four patients prior to tyrosine kinase inhibitor therapy (Pretreatment) and at time of overall best response during follow-up on therapy (Follow-up). According to Response Evaluation Criteria in Solid Tumors v. 1.1 response and change in target lesion diameter, the first two patients, JHRCC3 and JHRCC62, had partial and complete responses, respectively, in their target lesions. For the last two patients, JHRCC12 and JHRCC228, both had progression of disease in their target

lesions during therapy. Black arrow indicates the target lesion used in radiographic assessment. The targeted lesion was not the same lesion used for xenograft generation. CR = complete response; PD = progression of disease; PR = partial response; Ref = reference.

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Fig. 4.

Clinical and patient-derived xenograft treatment response in four patients treated with first-line sunitinib (Sun) for metastatic renal cell carcinoma. Tissues or cells used for xenograft assays include resected primary tumors (JHRCC3, JHRCC228), cultured cells from resected bone metastases (JHRCC12), and cultured cells from resected lymph node metastases (JHRCC62). In vitro treatment assays were performed on cell lines generated from patients' tumors (JHRCC12, JHRCC62, and JHRCC228) or a xenograft tumor (JHRCC3). (a) In vitro treatment results with Sun. Half maximal effective concentration curves generated from Cell Titer-Glo luminescent cell viability assay in four cell lines of patients; (b) in vivo treatment results with Sun. In each xenograft model, mice were randomized to a treatment group with Sun (60 mg/kg daily) or vehicle group with phosphate-buffered saline and monitored for tumor growth. At completion of treatment, mice were killed and tumors were harvested. Representative pictures from one mouse in each treatment arm among the four xenograft models are displayed. Photographed tumors were 27 d for JHRCC3, 30 d for JHRCC62 and

JHRCC12, and 21 d for JHRCC228. The p values were calculated using two-way analysis of variance.

C = cell passage number; EC₅₀ = half maximal effective concentration; M = mouse passage number; Sun = sunitinib; Veh = vehicle.

Table 1
Clinicopathologic characteristics of patients and specimens with corresponding
engraftment rates ($n = 74$)[†]

Characteristic	Total	Success	Failed
Patients, $n = 66$			
Age, yr, median (range)	56 (19–78)	52 (35–78)	59 (19–77)
Sex (%)			
Male	48	24 (50)	24 (50)
Female	18	5 (28)	13 (72)
Histology (%)			
Clear cell	45	19 (42)	26 (58)
Unclassified	8	4 (50)	4 (50)
Papillary	4	2 (50)	2 (50)
Translocation	3	2 (100)	–
Chromophobe	2	1 (50)	1 (50)
Epithelioid angiomyolipoma	2	–	2 (100)
Mucinous tubular and spindle cell carcinoma	1	1 (100)	–
Medullary	1	1 (100)	–
AJCC stage (%)			
Stage I	3	–	3 (100)
Stage II	2	–	2 (100)
Stage III	6	–	6 (100)
Stage IV	55	29 (53)	26 (47)
Specimens ($n = 74$)			
Biopsy specimens (%)			
Primary tumor	1	1 (100)	–
Metastatic tissue	11	6 (55)	5 (45)
Surgical resection specimens (%)			
Primary tumor	34	11 (32)	23 (68)
Metastatic tissue	28	15 (54)	13 (46)
Tissue (%)			
Primary tumor	35	12 (34)	23 (66)
Bone	16	10 (63)	6 (37)
Lymph node	5	2 (40)	3 (60)
Lung	4	1 (25)	3 (75)
Liver	4	2 (50)	2 (50)
Muscle	3	1 (33)	2 (67)
Adrenal	2	1 (50)	1 (50)
Perirenal	1	1 (100)	–
Bladder	1	1 (100)	–

Characteristic	Total	Success	Failed
Ascites [†]	1	1 (100)	–
Stomach	1	1 (100)	–
Omental Implant	1	–	1 (100)

AJCC = American Joint Committee on Cancer.

[†] Aspiration of ascites was included as a biopsy for the purpose of our analysis.