LABAZ1: A Metastatic Tumor Model for Renal Cell Carcinoma Expressing the Carboxic Anhydrase Type 9 Tumor Antigen


ABSTRACT

A metastatic renal cell carcinoma (RCC) tumor xenograft that expresses the targetable, membrane-bound tumor-associated antigen carboxic anhydrase type 9 (CA IX) is described. The xenograft, established from a high-grade type-2 chromophob RCC (cRCC), has been serially transplanted in immune compromised mice, in which it grows orthotopically under the renal capsule, doubling its size every 9 weeks and sending metastases to the lung and liver at ~20 weeks. Tumors were capable of being imaged using a micro-PET (micro-position emission tomograph) with an 18-fluorodeoxyglucose (18-FDG) tracer. Subsequent xenograft generations have conserved immunohistochemical and ultrastructural properties typical for malignant renal epithelium-derived neoplasia (vimentin+, CK-19+, CA IX+ with hypoxia-inducible factor (HIF)-1α constitutive expression) and have demonstrated extensive proliferation, lack of apoptosis, severe genetic alterations, and molecular expression alterations; transforming growth factor β1 (TGF-β1), hepatocyte growth factor (HGF), proto-oncogene (c-met), matrix metalloproteinase (MMP)-1, and vascular endothelial growth factor (VEGF) C and D were overexpressed, whereas human epidermal growth factor receptor (HER)-2, MMP-2 and MMP-9, VEGF-R3, p53, and p27 were severely down-regulated, suggesting a proangiogenic environment, local invasiveness, and facilitated lymphatic metastasis. Altogether, LABAZ1 provides a relevant and flexible model to study the biology of cRCC, the role of CA IX in RCC tumorigenesis, progression, and metastasis, and a platform for testing new targeted therapeutic strategies.

INTRODUCTION

Chromophil (papillary) RCC is diagnosed in 15% of RCC, the second most frequent in occurrence after the clear cell type. cRCC has been recognized as a distinct subtype of RCC based on histological and cytogenetic data (1). The existence of two different subtypes of cRCC has been proposed (2, 3): type 1, having small cells with pale cytoplasm and increased proportion of chromosome 7p and 17p gains (4); and type 2, having large eosinophilic cells, which occur more commonly in younger patients and which is associated with more aggressive characteristics (3) and with worse prognosis (2, 4, 5).

CA IX is recognized as a type III biomarker (6), meaning that it is still under investigation; it is induced constitutively in certain tumor types but is absent in normal tissues with the exception of gastric epithelium. It is highly expressed in RCC, presumably because of a frameshift mutation in the von Hippel-Lindau (VHL) gene complex (7–12). CA IX is thought to have a role in the regulation of hypoxia-induced cell proliferation and may be involved in oncogenesis and tumor progression (7, 13, 14). We have recently shown that high expression of CA IX is associated with improved prognosis in patients with metastatic clear cell RCC (15) but is associated with poor prognosis in patients with nonhereditary type II cRCC. Such opposite relationships between CA IX expression and prognosis have been shown before: poorer in cervical carcinoma (16), colorectal carcinoma (17), and esophageal cancer (18), and improved survival in lung cancer and breast cancer (19).

Reports on the establishment of RCC cell lines (20, 21) or tumor models (22–28) are available. However, the majority of these models are derived from cell lines and none are chromophil-type RCC or have made the distinction between type 1 and type 2 subgroups. To develop an orthotopic RCC xenograft tumor model in SCID mice that expresses the CA IX surface antigen, we have used tumor tissue from a patient with metastatic type 2 CA IX+ cRCC. This model should be useful for further investigation of the biology of type 2 cRCC and the implication of CA IX expression, as well as for testing CA IX-targeted and other therapeutic modalities.

MATERIALS AND METHODS

Human Tumor. The primary human tumor originated from a 70-year-old Caucasian female with a sporadic type 2 cRCC. The tumor was pathologically staged as T2N0M0 and graded 3 of 4 according to Fuhrman’s classification (Fig. 1). The patient received high-dose IL-2 immunotherapy 6 weeks after nephrectomy. At 3 years after treatment, the patient is alive with stable disease.

The specimen was opened under sterile conditions in the operating room. Fat, blood, and necrotic tissue were surgically removed. Small fragments of the fresh tumor tissue were cut and minced and reduced to a size of 2–4 mm and then immersed in RPMI 1640 at 4°C and transferred on ice under sterile conditions to the SCID mice colony for immediate transplantation. The study was approved by the Institutional Review Board (IRB) and the Experimental Animal Committee of the University of California, Los-Angeles.

Animal Mode. Fifty male 6–8 week-old CB17 white SCID mice were obtained from the breeding program at the Defined-Flora Pathogen-Free Mouse Colony at the University of California, Los Angeles, in five cohorts consisting of 10 mice/cohort. The mice were kept in a pathogen-free isolator unit. Food, water, and sawdust bedding were autoclaved, and the mice received chow and water ad libitum. The mice were anesthetized for survival surgery with a mixture of ketamine (2 mg) and xylazine (0.6 mg; both from Phoenix Scientific Inc., St. Joseph, MO) injected s.c. The site of incision was swabbed with 70% ethanol solution. The right kidney was exposed through a right flank incision and was partially exteriorized. Using a 27-gauge needle, we injected 100 μl of saline (0.9%) under the renal capsule to raise it from the underlying

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1 The abbreviations used are: RCC, Renal cell carcinoma; PET, positron emission tomography; 18-FDG, 18-fluorodeoxyglucose; CK, cytokeratin; TGF-β1, transforming growth factor β1; HGF, hepatocyte growth factor; MET, proto-oncogene (HGF receptor); MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; HER-2, human EGF-R2; CA IX, carbonic anhydrase type 9; IL, interleukin; SCID, severe combined immune deficient; CD10, cluster of differentiation 10; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; EGFR, epidermal growth factor receptor; bcl-2, B-cell lymphoma 2; PTEN, phosphatase and tensin homologue; STEAP, six-transmembrane epithelial antigen of the prostate; HIF, hypoxia-inducible factor; cRCC, chromophil (papillary) renal cell carcinoma.
Histology and Immunohistochemistry. Paraffin sections were cut at 3 μm and were baked overnight at 60°C. Slides were deparaffinized in xylene and rehydrated in graded ethyl alcohols series. Endogenous peroxidase activity was eliminated by treating slides with 3% hydrogen peroxide in methanol for 10 min. Heat-induced epitope retrieval was performed on the slides using 0.01 M citrate buffer and steaming at pH 6.0 in a vegetable steamer for vimentin, keratin 19, and keratin 20, using 0.001 M EDTA (pH 8.0) and a pressure cooker (Biocare Medical, Walnut Creek, CA) for Ki67. After heating (25 min for vegetable steamer and 3 min for pressure cooker), cooling and washing in 0.01 M PBS, slides were placed on a DAKO Autostainer (DAKO Corp, Carpenteria, CA) and then were sequentially incubated in primary antibody for 30 min, rabbit antimouse immunoglobulins (DAKO Corp) for 15 min, followed by DAKO Envision+ (rabbit, peroxidase; DAKO Corp) for 30 min. Diaminobenzidine and hydrogen peroxide were used as the substrates for the peroxidase enzyme. Primary antibodies included mouse monoclonal antibodies against vimentin (clone V9 at 1:1000 dilution), keratin 19 (clone RCK108 at 1:50 dilution), keratin 20 (clone K20.8 at 1:50 dilution), CD10, and Ki67 (clone MIB-1 at 1:100 dilution). For negative controls, normal mouse serum was used in place of the primary antibodies. All of the primary antibodies were obtained from DAKO Corp. For detection of CA IX expression, sectioned tissue was used in place of the primary antibodies. The injection resulted in the formation of a transparent subcapsular vesicle. Then, a small slit was made on the vesicle using the tip of the needle. The edge of the transparent elastic renal capsule was retracted on both sides and 3–4 fresh tumor fragments were implanted into the subcapsular space with 100 μl of Matrigel Matrix (Beckton-Dickenson Bioscience, Bedford, MA). The capsule was permitted to collapse and, therefore, fix the tumor fragments in close contact with the renal parenchyma. The kidney was then allowed to fall back into the abdominal cavity. The body wall and the skin incision were closed separately with absorbable 5–0 vicryl sutures (Ethicon INC, Somerville, NJ). Subsequent transfer of five xenograft generations was performed in the same manner. Immediate (1–3 days) postoperative mortality was 5 (10%) of 50. Animals were sacrificed at different time intervals, and the primary tumor and organ of interest for metastatic spread were fixed and paraffin embedded.

Fig. 1. Representative computerized tomography scan image demonstrating the renal tumor (solid arrow) and retroperitoneal lymphadenopathy (empty arrow) in the patient from whom LABAZ1 was derived.

Littleton, CO (Catalogue number NB 100-134). After antigen retrieval at 115°C in TRIS buffer (pH 9.00), sections were incubated with primary anti-serum diluted at 1:100 to 1:250. Localization was performed with the DAKO Envision+ kit followed by horseradish peroxidase (DAKO Corporation). The sections were counterstained with hematoxylin. For negative controls, normal rabbit serum was substituted for the primary antibody.

Sampled mouse xenograft tissues were fixed for 4 h in 10% buffered formalin and were then embedded in paraffin blocks. Immunohistochemical staining was performed using the DAKO Animal Research Kit (ARK). Serial sections were subject to heat antigen retrieval, as described above, and were incubated with the primary antibody diluted 1:100. The slides were incubated sequentially with biotin-conjugated antimouse antibody with added normal mouse serum. Detection was performed with horseradish peroxidase using the ARK kit. Cell proliferation was determined by Ki67 staining and was calculated by manually counting total epithelial nuclei and Ki67-expressing epithelial nuclei in five microscopic fields in the primary human tumor, LABAZ1 xenograft, and benign human or murine renal tissues to a total of 1000 nuclei for each. To assess apoptosis, we performed a TUNEL assay, as described previously (29). For both the Ki67 and the TUNEL tests, results were tested using ANOVA, with a P < 0.05 being considered significant.

Electron Microscopy. Tissue was obtained at the time of xenograft tumor resection and immediately fixed in gluteraldehyde for ultrastructural examination. Tissue processing for ultrastructural examination was carried out using methods for the embedding of specimens for electron microscopy that have been described previously (30). Briefly, specimens were fixed in gluteraldehyde, were postfixed for 1 h in osmium colloidine, and were embedded in epon.

Cytogenetics. Chromosomal analysis was performed on fresh primary tumor and xenografts. On receipt, the tissue was aseptically minced into 2–3-mm pieces and was digested with collagenase II (5 mg/ml/100 g tissue; Worthington Biochem, Freehold, NJ), as described elsewhere (31, 32). After tissue dissociation, cells were washed and then were cultured in RPMI 1640 supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin at a final concentration of 1–2×10⁵ cells/ml of medium in a T-25 flask. The cultures were harvested when growth was subconfluent and cells were actively dividing. Cells were subjected to hypotonic treatment (potassium chloride, 0.075 M) for 30 min at 37°C and were fixed in methanol and acetic acid (3:1). Chromosome analysis was performed on 20G-banding with trypsin and Giemsa (GTG)-banded metaphase cells from two or more cultures. Interpretation was made in accordance with International System for Human Cytogenetic Nomenclature (ISCN) guidelines (33). Numeric changes were
tabulated relative to specimen ploidy. Clonal abnormality was established only when two or more cells had the same extra chromosome, structural abnormality, or three or more cells with the same missing chromosome. 

**PET.** Tumor-bearing mice were imaged using a PET scanner specifically designed for small animal imaging (micro-PET) with 18-FDG 96 h after tumor transplantation and once animals were palpable. Mice were injected with 200–300 mCi of radiolabeled tracer, placed in a spread supine position, and scanned with the micro-PET (34, 35). Scanning was performed 60–90 min after tracer injection, to allow for clearance of background activity before starting image acquisition. Scanning was performed with the long axis of the mouse parallel to the long axis of the scanner, with the mouse in a prone position. Scanning conditions were identical to those described previously (36). Acquisition time was 56 min, 8 min/position, with seven bed positions. Images were reconstructed with filter-back projection and an iterative three-dimensional technique (37), with an isotropic image resolution of 1.8 mm and a volumetric resolution of ~8 mm³.

**Semiqualitative Reverse Transcriptase-PCR.** Total RNA was extracted from the primary human tumor, from resected retroperitoneal lymph nodes, xenograft, contralateral normal murine kidney, LNCaP cell line, and CL1 prostate cancer xenograft (38) as positive controls by acid guanidine isothiocyanate-phenol-chloroform and were reverse transcribed into cDNA. The amount of diluted cDNA that expressed equivalent signal intensity of β-actin was used to perform PCR for the other markers. The oligonucleotide primer pair sequences and cycle settings used for β-actin; p53; p27; E-cadherin; IL-6 and –8; VEGF-A, -B, -C, and -D; VEGF-R2 and -R3; EGFR; TGF-β1 and -β2; bcl-2; c-met; HGF; PTEN; STEAP; collagenase; MMP-2 and –9, and HER2 were as described previously (38, 39). The primer pair sequences for CA IX were 5′-CAA TAT GAG GGG TCT CTG ACTACA C and 3′-H11032 CAA TAT GAG GGG TCT CTG ACTACA C and the cycle settings consisted of 94°C for 2 min followed by 30 cycles (94°C at 1 min, 65°C at 2 min).

**RESULTS**

**Establishment of Tumor Model and Pattern of Growth**

Forty-five (90%) of 50 mice survived the immediate postoperative period. In the first xenograft generation, eight (80%) mice survived, of which six grew palpable tumors (xenograft take rate, 75%). Starting with LABAZ1 second generation, all of the mice surviving the postoperative period grew tumors (xenograft transfer take rate, 100%). At 96 h after subcapsular implantation of the xenograft particles, a reduced 18-FDG uptake was noted over the right kidney but an increased 18-FDG uptake was traced in the periphery of the right kidney corresponding with the subcapsular location of the transplanted LABAZ1 cells (Fig. 2).

The time to grow a 1-cm palpable tumor shortened progressively with each subsequent xenograft transfer (Table 1). One member of each xenograft generation cohort was sacrificed weekly after palpation of 1 cm. Tumor, except for the final two mice in each cohort that were observed until the primary tumor grew to a size mandating euthanasia (Fig. 3). LABAZ1 tumors are locally invasive into neighboring organs (Fig. 4, A and B). On the basis of macroscopic organ survey and histological examination of the lung, liver, bony skeleton, spleen, brain, and posterior peritoneal lymphatic tissue, the initial three cohorts did not metastasize (Table 1). Starting with the fourth generation, metastases were noted at 19–20 weeks after xenograft implantation. In generation 4 and 5, all of the mice sacrificed after week 19 and 20, respectively, had histological evidence of systemic metastasis to the lung and/or liver as well as peritoneum, and all had orthotopic tumors that had invaded into the liver as well as the small and large bowel. For generations 4 and 5, time to grow a 2-cm palpable tumor was 21 ± 2 weeks, approximately corresponding with the time to metastasis. Therefore, the doubling time of the tumor is estimated to be ~9 weeks in the final LABAZ1 generation. Four mice, kept on observation in generation 4 (n = 2) and 5 (n = 2), had to be sacrificed for failure to thrive at 22 ± 3 weeks, representing an approximated survival of 23 weeks after xenograft implantation.

**Histological and Immunohistochemical Characteristics**

The original primary human tumor consisted of papillae lined by epithelial cells with abundant eosinophilic cytoplasm arranged in a pseud stratified and irregularly stratified manner, compatible with the original description of type 2 cRCC by Delahunt et al. (Ref. 3; Fig. 5A).

Typical for RCC, the primary tumor also reacted positively with antibodies for vimentin and CK-19 (Fig. 5, B and C, respectively) and negatively for CK-20 (Fig. 5D). The primary human tumor was positive for membranal CA IX (Fig. 5E), whereas murine tissues were not (data not shown). This histological and immunohistochemical profile was reproduced in all LABAZ1 generations and shown for generation 5 only in a corresponding fashion in Fig. 5, F–J. The percentage of LABAZ1 xenograft cells staining positively for CD10 was 5 ± 1% (Fig. 5K) and for Ki67 36 ± 5% in the primary human tumor and 58 ± 3% in the LABAZ1 xenograft (P < 0.05; Fig. 5L). TUNEL staining did not demonstrate any significant apoptotic activity in the LABAZ1 xenograft (Fig. 5M).

**CA IX.** The primary human tumor stained positive for CA IX (Fig. 5E). The fifth generation LABAZ1 xenograft (Fig. 5J) and its metastatic lung (Fig. 5N) and liver deposits maintained a high level of membranal CA IX expression. LABAZ1 xenograft particles were processed into single-cell suspension pooled and frozen at −180°C for 8 weeks. After being defrosted, LABAZ1 cells maintained membranal CA IX expression (Fig. 5O) and were tumorigenic both s.c. and orthotopically. The

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**Table 1. Generation of LABAZ1 RCC tumor model in four transfers of xenografts in SCID mice**

<table>
<thead>
<tr>
<th>Xenograft generation</th>
<th>Time to 1-cm palpable tumor (wk)</th>
<th>Time to metastasis (wk)</th>
<th>Mice surviving surgery (tumor take)</th>
<th>Mice on observation sacrificed at (wk)</th>
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<tbody>
<tr>
<td>1</td>
<td>33 ± 3</td>
<td>none</td>
<td>8 (6)</td>
<td>62 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>21 ± 2</td>
<td>none</td>
<td>9 (9)</td>
<td>53 ± 1</td>
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<td>3</td>
<td>16 ± 2</td>
<td>none</td>
<td>9 (9)</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>12 ± 2</td>
<td>19</td>
<td>9 (9)</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>12 ± 2</td>
<td>20</td>
<td>10 (30)</td>
<td>23 ± 1</td>
</tr>
</tbody>
</table>

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Fig. 2. Micro-PET tomographic images using FDG as a PET reporter substrate of a mouse xenografted with LABAZ1. Arrows, the xenografted right kidney.
immunohistochemical profile of these reconstituted xenografts were identical to fifth generation LABAZ1 xenografts.

**HIF-1α.** The primary human tumor stained positive for HIF-1α (Fig. 5P). The fifth generation LABAZ1 xenograft and its metastatic lung metastasis (Fig. 5Q) maintained a high level of HIF-1α expression, indicating that the CA-IX expression is attributable to the constituted expression of HIF-1 and is not caused by the effect of *in vitro* hypoxia.

**Electron Microscopy**

The LABAZ1 demonstrated clusters of malignant cells with oval nuclei and nucleoli. The cytoplasm contained mitochondria, lipid droplets, glycogen, and occasional lysosomes. The cell surface contained microvilli processes as well as intracellular lumens lined by microvilous processes indicating tubular differentiation, all of which are typical for human malignant cells of epithelial origin (Fig. 6, A and

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**Fig. 3.** LABAZ1 orthotopic xenograph (A) gross appearance after euthanasia. B, micro-PET all-body imaging of xenografted SCID mouse before euthanasia; compare with (C) normal mouse. Image visualized with FDG as PET reporter substrate. K, kidney; L, liver; X, xenograft.

**Fig. 4.** Local invasiveness and metastatic potential of LABAZ1. A, a necropsy macroscopic view from the abdominal cavity to the chest demonstrating local extension into the diaphragm, corresponding with B, vimentin-positive tumor deposits infiltrate the muscle layers of the diaphragm. C and D, H+E micrograph demonstrating pulmonary metastasis strongly expressing vimentin (D). E, H+E micrograph of macroscopically depicted liver metastasis seen on the liver surface away from the primary tumor site. This lesion stains positive for vimentin (F). E1, inset, microscopically depicted liver metastasis situated inside the liver parenchyma.
B). All of the LABAZ1 generations demonstrated the same ultrastructural features (data not shown).

PCR results are summarized in Table 2. In comparison with the human primary tumor, LABAZ1 xenograft lost expression of HER-2, MMP-2 and MMP-9, VEGF-R3, p53, and p27 and had overexpression of TGF-β1, HGF, c-met, MMP-1, and VEGF-C and -D, relatively, to the primary human tumor and its lymph node metastases (except for TGF-β1).

**Cytogenetic Analysis**

The cytogenetic analysis of the primary human tumor showed a 46XX female karyotype with an abnormal chromosome analysis consistent with hypodiploid abnormal clones that have lost chromosomes 1, 3, 4, 6, 9, 11, 13, 14, 15, 18, 21, and 22 with a 1:13 translocation (34, X, −X, −1, −3, −4, −6, −9, −11, −13, der (13)t (1:13) (q21;q34), −14, −15, −16, −18, −21, −22 [cp18]/46, XX [2]). These features were consistent in the LABAZ1 xenografts except that the xenograft demonstrated a jumping translocation of the short arm of chromosome 1 from chromosome 13 in the primary tumor to chromosome 19 in the xenograft. The location of this translocation was maintained in the ensuing LABAZ1 generations (Fig. 7).

**DISCUSSION**

We have established an orthotopic human RCC xenograft model in SCID mice that has been designated LABAZ1. The xenograft produced tumor containing 46 chromosomes reproducibly, indicating it’s human origin. Xenografts show ultrastructural features of malignant epithelial cells with Ki67 staining showing active proliferation and markedly low apoptotic activity. The expression of STEAP (40) also supports the malignant nature of LABAZ1 cells. Histologically and immunohistochemically, all of the generations of LABAZ1 xenograft demonstrated properties typical for human RCC such as CA IX, vimentin, and cytokeratins expression as well as local invasion and...
distant metastatic spread to the lung and liver. The ultrastructural properties of the LABAZ1 xenograft supports its origin from type 2 cRCC. In a recent comparative study, Krishnan and Truong (41) have demonstrated that “eosinophilic” cRCC is characterized by variably sized microvilli, small amounts of cytoplasmic lipid, and increased number of mitochondria in the cytoplasm, all compatible with our TEM (transmission electron microscopy) findings in the LABAZ1 xenograft, except that we have found glycogen deposits in the LABAZ1, whereas Krishnan and Truong suggested that the presence of glycogen is typical for clear type RCC.

Molecular marker expression was also compatible with that previously reported for cRCC (Table 2), including CD10 expression (42), down-regulation of HER-2 (43), and overexpression of MMP-1 with down-regulation of MMP-2 and -9 (44). PTEN loss in clear cell RCC (45, 46) and allelic loss at 10q23.3 in chromophobe RCC (47) are considered a late-stage event and may contribute to the invasive and/or metastatic tumor phenotype. Because LABAZ1 express PTEN, it is probable that PTEN does not have a role in the aggressiveness of this chromophil xenograft.

The tumorigenicity of RCC xenografts correlates with poor clinical outcome (23). The high xenograft take rate of LABAZ1 points to its aggressiveness, which is further supported by overexpression of c-met. c-met has been recently shown to be associated with an aggressive phenotype in cRCC (48). LABAZ1 genetic instability, reflected by major chromosomal alterations, are, by far, more extensive than those previously described for cRCC (49), indicating on aggressive biological behavior. Other features that support aggressiveness are overexpression of the proangiogenic and lymphangiogenic factors IL-8 and VEGF-C and D, suggesting that tumor cells have the propensity to metastasize via the lymphatics (50). Overexpression of the immune inhibitory molecule TGF-β1 and the expression of E-cadherin, IL-6, EGFR, VEGF-R2, VEGF-A and -B, TGF-β2, and bcl-2 are further reflected in the local invasiveness of LABAZ1, its ability to interfere with cellular immune responses, and its ability to support its growth with angiogenesis. LABAZ1 has low expression of p27. Low p27 expression is reported to be a significant and independent unfavorable prognostic factor in patients with RCC and to correlate inversely with tumor aggressiveness (51). Conflicting evidences as to the role of p53 expression and the biology of chromophil RCC exists in the literature (52–54). The LABAZ1 has low expression of wild-type p53. Both p27 and p53 expression in the LABAZ1 xenograft support its biological aggressiveness and is in accord with the low apoptotic activity of the xenograft.

Translocations of chromosome 1 have been reported previously for cRCC, e.g., a t(X;1)(p11;q21) translocation (55–59). Positional cloning has demonstrated that, as a result of this translocation, the transcription factor binding to IGHM enhancer 3 (TFE3) gene on the X-chromosome becomes fused to a novel gene, PRCC, on chromosome 1. The small second exon of PRCC was found to be located adjacent to the t(X;1) breakpoint in the human gene on chromosome 1 and be responsible for the production of a protein with an unknown function. It may be possible that the jumping translocation of chromosome 1 to chromosome 13 in the primary human tumor to chromosome 19 in the LABAZ1 xenograft may be of biological importance and may exert a survival advantage for the xenograft. Further study is required to evaluate whether the association with chromosome 19 at a given break point is a random phenomenon or is an event of biological significance.

CA IX is the most significant tumor-associated molecular marker described for kidney cancer to date. Its expression is associated with significant changes in tumor biology. An immunohistochemical study demonstrated that CA IX is expressed in all types of RCCs, including chromophob RCC but not in chromophobe histology nor in benign renal lesions such as oncocytomas (12). We have recently shown that CA IX expression is highly associated with survivorship for kidney cancer. Low CA IX expression predicts a worse outcome for patients with locally advanced clear type RCC and is an independent predictor for poor prognosis in patients with metastatic clear type RCC (15). In 52 cRCC 65% were subclassified as type 2. The disease-specific survival of patients with type 2 chromophil RCC expressing CA IX, as an isolated group, was significantly inferior to those who were CA

<table>
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<th>Feature</th>
<th>Expression</th>
<th>Relative to human primary</th>
<th>Relative to human LN metastasis</th>
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<tr>
<td>HER-2</td>
<td>Negative</td>
<td>↑</td>
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<tr>
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<tr>
<td>VEGF-R2</td>
<td>Positive</td>
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Table 2. Summary of semiquantitative PCR of LABAZ1 xenograft sorted by expression level relatively to the original primary human tumor and to the human lymph node (LN) metastasis.
IX negative or type 1.\textsuperscript{4} Therefore, having a model that is CA IX positive with constitutive HIF-1\(\alpha\) expressing type 2 cRCC is important because it represents a platform to study an aggressive subtype of chromophil RCC that, furthermore, expresses a targetable marker on its cell membrane.

The tumor-doubling time, time to metastasis, and estimated survival of LABAZ1-bearing SCID mice are conserved within a narrow time range. As such, it enables phenotypic characterization and the development and preclinical evaluation of possible therapeutic modalities for metastatic RCC. Furthermore, the development of the LABAZ1 xenograft may be imaged in a micro-PET facility using 18-FDG tracer as early as 96 h after implantation. The expression of CA IX by the LABAZ1 can also be further exploited for noninvasive, RCC-specific molecular imaging using CA IX promoter activity (60) that drives the expression of reporter genes such as herpes simplex virus thymidine kinase SR39 (HSV-tk-SR39) for PET imaging (61) or luciferase for imaging with a charge-coupled device camera (62).

Such constructs are currently being developed.

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lesions: electron microscopy permits distinction from cytomegalovirus (CMV).
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Amnon Zisman, Allan J. Pantuck, Matthew H. T. Bui, et al.


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