ORTHOTOPIC TREATMENT MODEL OF PROSTATE CANCER AND METASTASIS IN THE IMMUNOCOMPETENT MOUSE: EFFICACY OF FLT3 LIGAND IMMUNOTHERAPY

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We established an orthotopic treatment model of prostate cancer to generate reproducible primary and metastatic carcinoma in immunocompetent C57BL/6 mice. Using an in vivo selection scheme of intraprostatic implantation of TRAMP-C1 cells, primary prostate tumors were cultured and recycled three times by intraprostatic injection resulting in the selection and establishment of the recycled cell line TRAMP-C1P3. Prostate tumors were detected 30 days post-implantation with periaortic lymph node metastasis in 19/20 (95%) of mice. Tissue culture amplification, DNA ploidy and PCR amplification of the SV40 transgene were used to detect metastatic TRAMP-C1P3 in lymph node specimens. Tissue culture amplification and DNA ploidy were as sensitive as SV40 transgene amplification by PCR in detection of early metastatic disease in draining lymph nodes. To establish the use of the orthotopic model of prostate cancer for immunotherapy, mice were injected orthotopically with TRAMP-C1P3 cells and 7 days post-implantation treated daily for 28 days with either FLT3L or carrier control. Carrier-treated mice had clinically detectable prostate tumors, lymph node metastasis and were moribund at 29–35 days, whereas FLT3L therapy markedly suppressed primary TRAMP-C1P3 growth and lymph node metastasis, and prolonged survival. In summary, we have established a reproducible and clinically relevant orthotopic treatment model of prostate cancer in immunocompetent mice with application to a variety of therapeutic strategies. We demonstrate that FLT3L treatment suppressed orthotopic prostate tumor growth and lymph node metastasis reinforcing a role for FLT3L as an immunotherapeutic strategy for prostate cancer.

Key words: prostate cancer; metastasis; orthotopic mouse model; FLT3 ligand; immunotherapy

Experimental animal models of cancer have provided invaluable information about the mechanisms of tumor development, progression and metastasis. Despite the high prevalence of prostate cancer and the burden of disease on society, few laboratory and preclinical models of prostate cancer have been developed that approximate the spectrum of tumor progression and metastatic disease. Notwithstanding improvements in the diagnosis and treatment of localized prostate cancer in recent years, metastatic disease presents a formidable challenge and is lethal for men with advanced prostate cancer. This has spurred efforts to understand the mechanisms responsible for the switch from the indolent to the metastatic tumor phenotype. Elucidation of the molecular pathways that regulate metastasis depends on the availability of clinically relevant in vivo animal models of prostate cancer that serve as laboratory surrogates to assess the efficacy of therapeutic or preventive modalities in vivo.

Xenograft models of prostate cancer that mimic features of the human disease have been developed in immunodeficient mice. These models have been critical in the identification of genes involved in tumor growth and progression and are employed extensively for testing novel drug, irradiation and emerging gene therapies. Moreover, orthotopic implantation of prostate tumor cells or tissue in nude mice resulted in the derivation of sublines with the capacity for enhanced tumor take and increased lymph node and distant metastasis. Transgenic and reconstitution models of prostate cancer have been used to study prostate carcinogenesis, metastasis, and to test novel therapeutic and chemoprevention regimens. The mouse prostate reconstitution (MPR) model utilizes fetal mouse urogenital sinus tissue that is infected ex vivo with recombinant retroviruses then implanted into mice. Transgenic mouse models of prostate cancer have been developed in which the rat probasin promoter targets prostate epithelial cell-specific expression of SV40 T antigen (Tag) leading to the development of spontaneous autochthonous prostate cancer that is regulated temporally and spatially. In the transgenic adenocarcinoma mouse prostate (TRAMP) model, all males develop primary prostate tumors and metastases to distant sites between 18–24 weeks of age.

We demonstrated previously that treatment of mice bearing ectopic transplantable TRAMP-C1 tumors with FLT3 ligand (L), a hematopoietic growth factor, resulted in transient tumor regression without long-term antitumor immunity. The establishment of an orthotopic model of primary prostate cancer and lymph node metastasis in immunocompetent mice. To establish the use of this model for immunotherapy, the therapeutic efficacy of FLT3L was assessed in mice with orthotopic, metastatic prostate cancer.

MATERIAL AND METHODS

Cell lines and culture

The parental transplantable epithelial prostate cancer cell line, TRAMP-C1, derived from a prostate adenocarcinoma in transgenic adenocarcinoma of the mouse prostate (TRAMP-C1) tumors with FLT3 ligand (L), a hematopoietic growth factor, resulted in transient tumor regression without long-term antitumor immunity. The establishment of an orthotopic model of primary prostate cancer and lymph node metastasis in immunocompetent mice. To establish the use of this model for immunotherapy, the therapeutic efficacy of FLT3L was assessed in mice with orthotopic, metastatic prostate cancer.

Abbreviations: TRAMP, transgenic adenocarcinoma of the mouse prostate; Tag, T antigen; PCR, polymerase chain reaction; PBS, phosphate buffered saline; NMS, normal mouse serum; H&E, hematoxylin and eosin; FLT3L, flms-like tyrosine kinase 3 ligand; DLN, draining lymph node.

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genic C57BL/6 mice, was kindly provided by Dr. N. Greenberg (Baylor College of Medicine, Houston, TX). The TRAMP-C1P3 cell line was derived after 3 cycles of culture and intraprostatic implantation. TRAMP-C1, TRAMP-C1P3 and lymph node tumor-derived cell lines were cultured in DMEM supplemented with 5% FBS, 5% NuSerum IV (Collaborative Biomedical Products, Bedford, MA), 5 μg/ml insulin and 10 nM dihydrotestosterone.

**Establishment of orthotopic prostate tumor model**

Seven- to eight-week-old C57BL/6 mice were purchased from Charles River Laboratories, Wilmington, MA and experiments carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Mice anesthetized by isoflurane inhalation carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Mice anesthetized by isoflurane inhalation were injected in the right posterior prostatic lobe through a midline incision. TRAMP-C1P3 cells were injected into the prostates of C57BL/6 mice and primary prostate tumors isolated, grown in tissue culture and injected similarly into additional mice. After three cycles in vivo, the derived TRAMP-C1P3 cell line consistently produced orthotopic tumors with metastases to lymph nodes and extranodal sites. Orthotopically derived TRAMP-C1P3 cell line was compared to parental TRAMP-C1 for DNA ploidy by flow cytometry.

**Histological analysis**

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and 5 μm sections stained with H&E. Images were captured using a compound microscope and were assembled with Adobe Photoshop and PowerPoint software.

**Flow cytometry analysis of DNA content**

Primary tumors dissociated in collagenase buffer or derived cell lines dissociated by trypsinization were fixed in cold 70% ethanol and stained with 0.5 ml propidium iodide (PI, 50 μg/ml; Sigma, St. Louis, MO) and 100 μg/ml RNase A (100 U/ml; Sigma) for 30 min to overnight at 4°C and analyzed on a BD FACScan cytometer. DNA histograms were created using MODFIT software. Mouse splenocytes were used as an internal diploid control by staining with PI, 50 μg/ml.

**DNA extraction and PCR**

DNA was extracted from mouse prostate and lymph node tissue using the Genomic Prep cells and tissue DNA isolation kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer’s instructions. In some cases as indicated, the DNA was dialyzed against TRIS, EDTA (pH 8) solution for 30 min to remove possible low molecular weight inhibitors that could interfere with the PCR reaction. Two sets of primers were used to amplify the SV40 large T antigen (Tag). A 1353-bp target was amplified using the outer primers (forward, 5’-CTG GCC TGC AGT GTT TTA GGC ACA CTG-3’; reverse, 5’-GAT GAA TGG GAG CAG CAG TGG TGG TGG AAT GCC T-3’) (Invitrogen, Carlsbad, CA). A 655-bp target was amplified using nested (n) primers (forward, 5’-CTG GTC AAT AGC TAC TCC TAG C-3’; reverse, 5’-CCA GGG TTG AAG GAG CAT GAT-3’) (Integrated DNA Technologies, Coralville, IA). Amplification was carried out with 100 ng template DNA using the TaqPCR kit (BD Biosciences Clontech, Palo Alto, CA) in a total volume of 50 μl containing 3.5 mM MgCl₂ and 0.5 μM of each primer set based on the manufacturer’s instructions. PCR conditions were 38 cycles at 95°C for 30 sec, 68°C for 45 sec and a 105 sec final extension at 68°C (outer primers) or 35 cycles at 95°C, 45 sec 68°C, and a 105 sec final extension at 68°C (nested primers). For sequential PCR using n-SV40 primers, the PCR product of the first round using outer SV40 primers was cleaned up using QIAquick PCR purification kit (Qiagen, Valencia, CA) based on the provided protocol and the DNA was eluted in 30 μl of elution buffer. Dilutions of the eluted DNA were used in the second PCR reaction using n-SV40 primers. In parallel, the β-globin gene was used as an internal control for sample sufficiency. A 494-bp target was amplified using the following primers: (forward, 5’-CCA ATC TGC TCA CAC AGG ATA GAG AGG GCA GG-3’; reverse, 5’-CTG TGA GGC TGT CCA AGT GAT TCA GGC CAT CG-3’) (Integrated DNA Technologies). PCR products were separated on a 1% agarose gel and visualized with GelStar nucleic acid gel stain (Bio-Whittaker Molecular Applications, Rockland, ME).

**In vivo Flt3L therapy**

Flt3L was provided by ImmuneX Corporation (Seattle, WA) and diluted in 0.1% normal mouse serum (NMS) in PBS. Mice were injected orthotopically with TRAMP-C1P3 cells. Seven days post-implantation, mice were randomly assigned for 28 consecutive days of treatment by s.c. injection with either Flt3L (30 μg/injection) or carrier (0.1% NMS in PBS) as described previously. Mice were sacrificed after 28 days of therapy or when moribund to assess primary and metastatic tumor growth. Tumor tissue was evaluated by histopathology and flow cytometry.

**RESULTS**

**Establishment of the orthotopic model of prostate cancer**

An orthotopic model of prostate cancer was established in immunocompetent C57BL/6 mice using an in vivo selection scheme shown in Figure 1. Intraprostatic injection of 5 × 10⁶ TRAMP-C1 cells resulted in tumors in the prostate that were clinically palpable between 57–76 days after the initial injection. Prostate tumors were cultured and recycled 3 times by intraprostatic injection resulting in the in vivo selection and establishment of the recycled cell line TRAMP-C1P3. After 3 successive cycles in vivo, TRAMP-C1P3 cells produced histologically detectable tumor in the prostate at 14 days and clinically detectable tumors after a latent period of ~30 days; mice bearing prostate tumors...
were moribund by 30–35 days post-implantation of TRAMP-C1P3 cells. In 5 independent experiments, prostate tumors were detected clinically and confirmed histologically in 20 of 20 mice at ~1 month post-implantation with a range of 29–35 days. The orthotopic surgical technique employed was highly successful with 98% animal survival. Orthotopic injection of TRAMP-C1P3 cells resulted in reproducible prostate tumors with lymph node metastasis and occasional metastasis to extranodal sites (Fig. 2a). At autopsy, metastasis to periaortic lymph nodes was observed at high frequency with less common local tumor growth and metastasis to seminal vesicles, bladder and adrenals, as well as distant metastasis to the spleen, mesenteric lymph nodes, diaphragm and intestine. TRAMP-C1P3 tumors did not metastasize to bone as assessed by gross examination of skeletal bone at necropsy. Histologically, TRAMP-C1P3 tumors growing in the prostate were highly anaplastic often replacing the normal glandular architecture (Fig. 2b). Histological evaluation of lymph node and distant metastases revealed focal TRAMP-C1P3 tumor growth in periaortic lymph nodes (Fig. 2c) and tumor invading the small bowel (Fig. 2d).

**Characterization of TRAMP-C1P3 lymph node metastasis**

The parental TRAMP-C1 prostate cancer cell line used to develop the orthotopic model no longer expresses SV40 Tag protein or mRNA, or other prostate-specific tumor markers that could be used to detect disseminated tumor at secondary sites. To improve the sensitivity to detect TRAMP-C1P3 lymph node metastases, we took advantage of the in vitro cellular immortality and aneuploid DNA content of TRAMP-C1P3 cells. Clinically enlarged or suspicious lymph nodes from mice with orthotopic prostate tumors were excised and cultured in vitro as described in the Material and Methods. Tumor cells migrated out from the lymph node explants, proliferated, were expanded as cell lines, and analyzed by flow cytometry for DNA content. Figure 3 demonstrates an aneuploid DNA content of 1.7 and 1.5 for TRAMP-C1 and TRAMP-C1P3, respectively (Fig. 3a,b). Examples of cell lines derived from 2 independent lymph node metastases are shown in Figures 3c,d with DNA indices of 1.65 and 1.55. Using this tissue culture approach, metastatic TRAMP-C1P3 tumor was detected in lymph nodes cultured from 19/20 control (carrier treated) mice in five independent experiments (Table I). To compare the sensitivity of

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**FIGURE 2** - Orthotopic growth and metastasis of TRAMP-C1P3 cells in C57BL/6 mice after orthotopic implantation and histology of TRAMP-C1P3 tumors. (a) Local tumor growth in prostate (black arrow); seminal vesicles (white arrow), mesenteric tumor nodules (short white arrow), intestinal metastasis (short black arrow) and abdominal wall metastases (black arrowhead). (b) Tumor cells (black arrows) infiltrating between mouse prostatic glands (white arrow) (H & E staining; bar = 150 μm). (c) Subcapsular periaortic lymph node metastasis of TRAMP-C1P3 tumor (black arrow). Lymph node capsule (white arrow). (H & E staining; bar = 38 μm). (d) TRAMP-C1P3 tumor (black arrows) metastasis and invasion of the small bowel. Normal smooth muscle (white arrow) (H & E staining; bar = 375 μm).
tissue culture and DNA ploidy analysis with histopathology in detection of metastasis, a series of 5 lymph node specimens were split and analyzed by each technique. Three of five (60%) lymph node specimens that were negative by histopathology were aneuploid by flow cytometry after expansion in culture and analysis of DNA content. To rule out the possibility that aneuploid cell lines derived from lymph node or other tissues could be explained by spontaneous transformation of normal mouse cells in vitro, we determined that cells cultured from normal mouse prostate, bladder, spleen and lymph nodes had limited proliferative capacity and a finite life span in vitro usually undergoing cell senescence after 1 or 2 serial passages in culture. In those cases where sufficient cells were obtained for ploidy analysis, the DNA content was consistently diploid (data not shown).

Kinetics of TRAMP-C1P3 prostate tumor growth and lymph node metastasis

As a complementary and potentially more sensitive method for detection of TRAMP-C1P3 cells in the prostate or draining lymph nodes (DLNs) of mice injected orthotopically, genomic DNA from prostate and LN tissues was screened for the SV40 transgene by PCR. Initially, we determined that PCR products of 1353 bp (Fig. 4a, lane 2) and 655 bp (lane 4) were detected in TRAMP-C1P3 genomic DNA using 2 sets of primers for SV40 Tag. Specific SV40 Tag DNA sequences were verified by NdeI digestion yielding DNA fragments of the expected size (Fig. 4a, lanes 3, 5). To determine if the SV40 transgene was a reliable marker for detection of TRAMP-C1P3 cells, 12 independent single cell clones of TRAMP-C1P3 were isolated by limiting dilution, DNA extracted from each and analyzed by PCR. Genomic DNA from all clones was positive for SV40 Tag by PCR (Fig. 4b) providing evidence for the monoclonality of the SV40 transgene in the TRAMP-C1P3 cell line. To assess the sensitivity of PCR to detect the SV40 transgene, TRAMP-C1P3 cells were mixed with lymph node-derived lymphocytes to achieve the following ratios of TRAMP-C1P3 to lymphocytes: 1/5, 1/50, 1/500, 1/5,000, 1/50,000, and 1/500,000. DNA was extracted from the mixtures and analyzed by sequential PCR. TRAMP-C1P3 cells comprising as few as 1/50,000 (0.0002%) of the mixture were detectable by PCR (Fig. 4c).
To compare the sensitivity between tissue culture amplification and PCR and to determine the kinetics of metastatic spread to DLNs during early stages of tumor progression, we implanted TRAMP-C1P3 orthotopically and 7 and 14 days later euthanized mice to recover prostate glands and DLNs. Mice were randomized into 2 groups and examined for the presence of TRAMP-C1P3 by either PCR or in vitro culture. SV40 DNA was detected in the prostates of 4 of 5 mice at Day 7 (Fig. 5a, lanes 1–5) and 5 of 5 mice at Day 14 (Fig. 5c, lanes 1–5) but not in normal prostate (Fig. 5a, lanes 7 and 6, respectively). Sequential PCR using nested primers detected SV40 DNA in the one animal at Day 7 that was negative using each of the single primer pairs (Fig. 5a, inset). In contrast, no SV40 DNA was detected by sequential PCR in DLNs of 5 mice at Day 7 (Fig. 5b, lanes 1–5), but was detected in 2 of 5 mice at 14 days (Fig. 5d, lanes 3, 4) post-injection under conditions where all samples were positive for β-globin DNA. In parallel, cohorts were sacrificed at 7 and 14 days post-injection, prostates and DLNs excised, and explant-derived tissue cultures established. Prostate and LN-derived cultures were analyzed for DNA ploidy by flow cytometry and SV40 Tag by PCR (Table II). Cells were successfully cultured from prostate glands of 4 of 4 mice 7 days after orthotopic implantation of TRAMP-C1P3 cells.
Three of the four prostate derived cultures (Pr 4-Pr6) were aneuploid and positive for the SV40 transgene by PCR. One prostate-derived culture at Day 7 (Pr2) grew slowly, had a near diploid DNA index (DI = 1.21) and undetectable SV40 transgene suggesting a normal prostate cell phenotype. Similarly, 3 of 4 cell lines (Pr2-Pr4) established from prostate glands of mice 14 days after TRAMP-C1P3 orthotopic implantation were aneuploid and SV40 transgene positive (Table II). The other culture (Pr1) grew slowly, had a DI of 1.19 and evinced replicative senescence.

TRAMP-C1P3 cells were isolated from the lymph nodes of 1 of 6 mice (LN4) 7 days after prostate injection and from 2 of 4 mice (LN2, LN4) 14 days after intra-prostatic implantation (Table II). Minimal or no outgrowth was observed in LN cultures from the other mice. Taken together, the results demonstrate that TRAMP-C1P3 cells were detected by PCR in prostate tissue of all mice 7 and 14 days after orthotopic injection, whereas viable TRAMP-C1P3 cells, assessed by in vitro growth, were isolated in prostate tissue in 3 (75%) of 4 mice at both 7 and 14 days. The results also indicate that TRAMP-C1P3 cells began to metastasize to DLNs on Day 7 (17% incidence), increased to 50% by Day 14 and progressed to 100% by 5 weeks (Table I and data not shown).

**Efficacy of flt3L immunotherapy in the orthotopic treatment model of prostate cancer**

To assess the in vivo efficacy of flt3L treatment of mice bearing established orthotopic tumors, mice were injected orthotopically with TRAMP-C1P3 cells. Seven days post-implantation, mice were treated daily for 28 days with either flt3L (30 μg) or carrier control. Mice were monitored daily for clinically palpable prostate tumors, sacrificed when moribund and evaluated for prostate tumors and lymph node metastasis. Flt3L treatment delayed appearance of prostate tumor (35–77 days) vs. carrier treatment (29–35 days) and suppressed metastasis to DLNs in 8 of 9 mice at 35 days post-implantation. Interestingly, most flt3L-treated mice that developed prostate tumors with delayed kinetics did not have evidence of lymph node metastasis. As shown in Figure 6, the survival of orthotopic tumor-bearing mice treated with flt3L was significantly longer compared to control mice treated with carrier (p = 0.008, log-rank test). In a repeat experiment, flt3L therapy reproducibly prolonged survival but no animals survived beyond 84 days.

**DISCUSSION**

A goal of the present study was to establish an orthotopic therapeutic model of prostate cancer in the immunocompetent mouse that would provide an animal model to evaluate antitumor immunity and immunotherapeutic strategies. Using an in vivo selection scheme of intraprostatic implantation of TRAMP-C1 cells, we developed an orthotopic model of prostate cancer that recapitulates many of the clinical and pathogenic features of human prostate cancer. Notably, the orthotopically-derived tumor TRAMP-C1P3 exhibited rapid locoregional spread and metastasis to lymph nodes and visceral organs. TRAMP-C1P3 tumor cells seeded regional periaortic lymph nodes in 95% of mice. The less frequent metastasis to distant organs presumably reflects rapid local tumor growth and spread with animals moribund ∼30 days post-implantation. A distinct advantage of the orthotopic TRAMP-C1P3 model is the reproducible development of prostate cancer and lymph node metastasis in a relatively short time period (∼30 days), a feature particularly useful for testing various therapeutic strategies. In contrast, primary prostate tumors and metastases develop stochastically over an 18–30-week time period in the TRAMP model, a characteristic that limits its use as a cost-effective, proof-of-principle treatment model.

Preferential metastatic spread of prostate cancer to lymph nodes and bone is the main cause of morbidity among prostate cancer patients. Bone metastasis was not detected by gross inspection nor were clinical signs of paraplegia observed in the model. Bone metastasis in transgenic models of prostate cancer is also an infrequent and age-dependent event occurring in mice ≥6 months of age.12,17,18 Moreover, there are isolated reports of xenograft models showing prostate tumor metastasis to bone.6,7 Recent reports demonstrated that human prostate cancer cells injected i.v. preferentially metastasized to human bone engrafted in humanized severe combined immunodeficient (SCID-hu) mice but not to either engrafted human lung, engrafted murine bone or native bone.

**FIGURE 6** – Survival of mice bearing orthotopic prostate tumors after treatment with flt3L or carrier. Mice were injected intraprostatically with TRAMP-C1P3 cells and 7 days later treated with either flt3L (30 μg) or carrier daily for 28 days. The first and second arrows indicate when carrier/flt3L treatment was initiated and terminated, respectively. Animals were evaluated daily for tumor and sacrificed when moribund. Open circles, carrier treated; closed circles, flt3L treated. Flt3L treatment significantly prolonged survival compared to carrier treatment group (p = 0.008, log-rank test).

**TABLE II – PHENOTYPE OF PROSTATE- AND LYMPH NODE METASTASIS-DERIVED CELL LINES**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue origin</th>
<th>Day post implantation</th>
<th>Ploidy (DNA index)1</th>
<th>SV40 transgene2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr2</td>
<td>Prostate</td>
<td>7</td>
<td>Diploid (1.21)</td>
<td>–</td>
</tr>
<tr>
<td>Pr4</td>
<td>Prostate</td>
<td>7</td>
<td>Aneuploid (1.89)</td>
<td>+</td>
</tr>
<tr>
<td>Pr5</td>
<td>Prostate</td>
<td>7</td>
<td>Aneuploid (1.77)</td>
<td>+</td>
</tr>
<tr>
<td>Pr6</td>
<td>Prostate</td>
<td>7</td>
<td>Aneuploid (2.04)</td>
<td>+</td>
</tr>
<tr>
<td>LN4</td>
<td>Lymph node</td>
<td>7</td>
<td>Aneuploid (1.67)</td>
<td>+</td>
</tr>
<tr>
<td>Pr1</td>
<td>Prostate</td>
<td>14</td>
<td>Diploid (1.19)</td>
<td>ND3</td>
</tr>
<tr>
<td>Pr2</td>
<td>Prostate</td>
<td>14</td>
<td>Aneuploid (2.07)</td>
<td>+</td>
</tr>
<tr>
<td>Pr3</td>
<td>Prostate</td>
<td>14</td>
<td>Aneuploid (2.0)</td>
<td>+</td>
</tr>
<tr>
<td>Pr4</td>
<td>Prostate</td>
<td>14</td>
<td>Aneuploid (1.84)</td>
<td>+</td>
</tr>
<tr>
<td>LN2</td>
<td>Lymph node</td>
<td>14</td>
<td>Aneuploid (1.88)</td>
<td>+</td>
</tr>
<tr>
<td>LN4</td>
<td>Lymph node</td>
<td>14</td>
<td>Aneuploid (1.91)</td>
<td>+</td>
</tr>
<tr>
<td>TRAMP-C1P3</td>
<td>Prostate</td>
<td>14</td>
<td>Aneuploid (1.50)</td>
<td>+</td>
</tr>
<tr>
<td>Splenocytes</td>
<td></td>
<td></td>
<td>Diploid</td>
<td></td>
</tr>
</tbody>
</table>

1 Determined by PI staining and flow cytometry. 2 PCR amplification of SV40 Tag using either single primer pairs or sequential PCR. 3 ND, not determined.
murine bone. These findings indicate that prostate cancer metastasis to bone is both species- and tissue-specific and may account for the limited reports of bone metastasis in conventional xenograft models. Taken together, these studies emphasize that transgenic mouse or xenograft models of prostate cancer may mimic but do not exactly reproduce the complete spectrum of prostate cancer thus warranting further development of animal models that recapitulate prostate tumor metastasis to bone.

Our data support the concept that the prostate microenvironment (soil) provides a niche for selective evolution of aggressive prostate tumors with metastatic potential. The lack of expression of T antigen or other prostate tumor markers by TRAMP-C1 or TRAMP-C1P3 cells prompted us to detect lymph node metastases by selective growth and amplification of tumor cells in tissue culture and identification by chromosomal instability, 2 hallmarks of the tumor cell phenotype. We further detected the SV40 transgene by PCR in 12 independently derived clones of TRAMP-C1P3 demonstrating that the transgene is an excellent tumor marker for this cell line. PCR was used to confirm the identity of aneuploid tissue culture outgrowths and also to compare the sensitivity of these 2 techniques to detect early metastatic disease. Sequential tissue culture amplification was more sensitive than amplification with a single set of primers. Detection of 1/50,000 TRAMP-C1P3 cells was achieved with this approach and samples negative by single primers plus amplification were positive by sequential PCR. Despite this level of sensitivity, tissue culture amplification, but not sequential PCR, detected metastatic TRAMP-C1P3 cells in DLN s on Day 7 indicating an unexpected level of sensitivity by this approach. These data also indicate that TRAMP-C1P3 cells have a high autonomous growth potential, a view consistent with limiting dilution analysis (data not shown). Although the overall sensitivity of these 2 approaches to detect metastatic clones was similar, distinct advantages/disadvantages exist with each method. For example, tissue culture amplification of aneuploid cells demonstrated unequivocally that TRAMP-C1P3 were viable when isolated, whereas this cannot be stated with certainty with PCR. Culture amplification required significantly more time (3–4 weeks) to obtain results.

We tested the efficacy of flt3-L treatment in an orthotopic metastatic TRAMP-C1P3 model, a clinically more relevant setting. Flt3-L is an attractive candidate for prostate cancer immunotherapy because it induces a marked inflammatory cell infiltrate into tumors. We reasoned that an enhanced tumor-specific immune response would not be sufficient for successful treatment of prostate cancer because of its propensity to lose expression of MHC Class I antigens during tumor progression. The enhanced innate immune response induced by flt3-L should eliminate MHC-negative variants that arise during tumor progression. Consistent with this view, flt3-L was observed to induce impressive tumor growth inhibition in a TRAMP-C1 ectopic treatment model despite rapid downregulation of Class I antigens in vivo. In the orthotopic model, Flt3L treatment also markedly suppressed primary TRAMP-C1P3 tumor growth and lymph node metastasis and prolonged survival after completion of therapy. Despite this impressive tumor growth inhibition, disease relapse was also a common feature in the orthotopic model. Although flt3-L has been shown to boost the anti-tumor immune response in the ectopic TRAMP-C1 model as well as other tumor models, therapeutic failure suggests that a sufficient anti-tumor immune response was not induced by flt3-L treatment. This was not due to the absence of intratumoral dendritic cells (DCs) or the absence of a tumor antigen on TRAMP-C1P3 cells. Flt3-L induced a potent prostatic DC infiltrate and TRAMP-C1 tumor cells expressed high levels of the Wilms’ tumor (WT1) antigen (unpublished observations). Treatment failure most likely reflected the immunosuppressive tumor microenvironment. Support for this possibility comes from our observation that intratumoral DCs are remarkably deficient in Class II antigen expression. In addition, the TCR/CD3 signaling complex was markedly disrupted on tumor-associated T cells, but not peripheral T cells (manuscript submitted). Thus, these observations suggest that several tumor escape mechanisms are operative within TRAMP-C1P3 tumors that inhibit development of anti-tumor immunity and prevent curative responses.

In conclusion, a reproducible and clinically relevant orthotopic treatment model of prostate cancer in immunocompetent C57BL/6 mice has been established that has application to a variety of therapeutic strategies. The model offers the opportunity to characterize the metastatic tumor phenotype and to identify by gene expression profiling gene products whose expression or silencing may contribute to prostate tumor metastasis. In turn, gene expression profiling may also be used to identify potential diagnostic markers and targets for imaging and therapy.

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REFERENCES

2. Yoshida BA, Sokoloff MM, Welch DR, Rinker-Schaeffer CW. Measurement of gene expression in tissue culture outgrowths and also to compare the sensitivity of these 2 techniques to detect early metastatic disease. Sequential tissue culture amplification was more sensitive than amplification with a single set of primers. Detection of 1/50,000 TRAMP-C1P3 cells was achieved with this approach and samples negative by single primers plus amplification were positive by sequential PCR. Despite this level of sensitivity, tissue culture amplification, but not sequential PCR, detected metastatic TRAMP-C1P3 cells in DLN s on Day 7 indicating an unexpected level of sensitivity by this approach. These data also indicate that TRAMP-C1P3 cells have a high autonomous growth potential, a view consistent with limiting dilution analysis (data not shown). Although the overall sensitivity of these 2 approaches to detect metastatic clones was similar, distinct advantages/disadvantages exist with each method. For example, tissue culture amplification of aneuploid cells demonstrated unequivocally that TRAMP-C1P3 were viable when isolated, whereas this cannot be stated with certainty with PCR. Culture amplification required significantly more time (3–4 weeks) to obtain results.

We tested the efficacy of flt3-L treatment in an orthotopic metastatic TRAMP-C1P3 model, a clinically more relevant setting. Flt3-L is an attractive candidate for prostate cancer immunotherapy because it induces a marked inflammatory cell infiltrate into tumors. We reasoned that an enhanced tumor-specific immune response would not be sufficient for successful treatment of prostate cancer because of its propensity to lose expression of MHC Class I antigens during tumor progression. The enhanced innate immune response induced by flt3-L should eliminate MHC-negative variants that arise during tumor progression. Consistent with this view, flt3-L was observed to induce impressive tumor growth inhibition in a TRAMP-C1 ectopic treatment model despite rapid downregulation of Class I antigens in vivo. In the orthotopic model, Flt3L treatment also markedly suppressed primary TRAMP-C1P3 tumor growth and lymph node metastasis and prolonged survival after completion of therapy. Despite this impressive tumor growth inhibition, disease relapse was also a common feature in the orthotopic model. Although flt3-L has been shown to boost the anti-tumor immune response in the ectopic TRAMP-C1 model as well as other tumor models, therapeutic failure suggests that a sufficient anti-tumor immune response was not induced by flt3-L treatment. This was not due to the absence of intratumoral dendritic cells (DCs) or the absence of a tumor antigen on TRAMP-C1P3 cells. Flt3-L induced a potent prostatic DC infiltrate and TRAMP-C1 tumor cells expressed high levels of the Wilms’ tumor (WT1) antigen (unpublished observations). Treatment failure most likely reflected the immunosuppressive tumor microenvironment. Support for this possibility comes from our observation that intratumoral DCs are remarkably deficient in Class II antigen expression. In addition, the TCR/CD3 signaling complex was markedly disrupted on tumor-associated T cells, but not peripheral T cells (manuscript submitted). Thus, these observations suggest that several tumor escape mechanisms are operative within TRAMP-C1P3 tumors that inhibit development of anti-tumor immunity and prevent curative responses.

In conclusion, a reproducible and clinically relevant orthotopic treatment model of prostate cancer in immunocompetent C57BL/6 mice has been established that has application to a variety of therapeutic strategies. The model offers the opportunity to characterize the metastatic tumor phenotype and to identify by gene expression profiling gene products whose expression or silencing may contribute to prostate tumor metastasis. In turn, gene expression profiling may also be used to identify potential diagnostic markers and targets for imaging and therapy.

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