

An orthotopic metastatic prostate cancer model in SCID mice via grafting of a transplantable human prostate tumor line

Yuwei Wang¹, Hui Xue¹, Jean-Claude Cutz^{1,2}, Jane Bayani³, Nasrin R Mawji⁴, Wilfred G Chen⁵, Lester J Goetz⁶, Simon W Hayward⁷, Marianne D Sadar⁴, C Blake Gilks⁸, Peter W Gout¹, Jeremy A Squire³, Gerald R Cunha⁹ and Yu-Zhuo Wang¹

¹Department of Cancer Endocrinology, BC Cancer Agency, Vancouver, British Columbia, Canada;

²Department of Anatomical Pathology, St Joseph's Hospital—Hamilton Health Sciences, Hamilton, Ontario, Canada;

³Department of Laboratory Medicine and Pathobiology, Ontario Cancer Institute, Toronto, Ontario, Canada;

⁴Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, British Columbia, Canada;

⁵Urology Research Unit, Carlton Centre, San Fernando, Trinidad;

⁶Department of Urology, Gulf View Medical, La Romaine, Trinidad;

⁷Departments of Urologic Surgery and Cancer Biology, Vanderbilt University Medical Center, Nashville, TN, USA;

⁸Department of Pathology, Vancouver General Hospital, University of British Columbia, Vancouver, British Columbia, Canada and ⁹Departments of Anatomy and Urology, University of California San Francisco, San Francisco, CA, USA

Metastasis is the major cause of prostate cancer deaths and there is a need for clinically relevant *in vivo* models allowing elucidation of molecular and cellular mechanisms underlying metastatic behavior. Here we describe the development of a new *in vivo* model system for metastatic prostate cancer. Pieces of prostate cancer tissue from a patient were grafted in testosterone-supplemented male NOD-SCID mice at the subrenal capsule graft site permitting high tumor take rates. After five serial transplantations, the tumor tissues were grafted into mouse prostates. Resulting tumors and suspected metastatic lesions were subjected to histopathological and immunohistochemical analysis. Samples of metastatic tissue were regrafted in mouse anterior prostates and their growth and spread examined, leading to isolation from lymph nodes of a metastatic subline, PCa1-met. Orthotopic grafting of PCa1-met tissue in 47 hosts led in all cases to metastases to multiple organs (lymph nodes, lung, liver, kidney, spleen and, notably, bone). Histopathological analysis showed strong similarity between orthotopic grafts and their metastases. The latter were of human origin as indicated by immunostaining using antibodies against human mitochondria, androgen receptor, prostate-specific antigen and Ki-67. Spectral karyotyping showed few chromosomal alterations in the PCa1-met subline. This study indicates that transplantable subrenal capsule xenografts of human prostate cancer tissue in NOD-SCID mice can, as distinct from primary cancer tissue, be successfully grown in the orthotopic site. Orthotopic xenografts of the transplantable tumor lines and metastatic sublines can be used for studying various aspects of metastatic prostate cancer, including metastasis to bone.

Laboratory Investigation (2005) **85**, 1392–1404. doi:10.1038/labinvest.3700335; published online 12 September 2005

Keywords: human prostate cancer; metastatic model; orthotopic; xenograft; subrenal capsule

Metastatic prostate cancers have a poor prognosis with limited responses to palliative therapies such as androgen ablation and chemotherapy.^{1,2} The development of new, effective therapies for metastatic prostate cancer is considered to be critically

dependent on the availability of clinically relevant *in vivo* models.³ Current models used for studying prostate cancer metastasis and drug evaluation generally consist of xenografts in immuno-deficient mice of well-established human prostate cancer cell lines that have adapted to *in vitro* growth, for example, LNCaP and PC-3.^{4–6} Such models have been useful for identifying cellular and molecular mechanisms underlying metastasis and development of new therapeutics. However, they have profound shortcomings: the highly anaplastic

Correspondence: Dr Y-Z Wang, PhD, Department of Cancer Endocrinology, BC Cancer Agency—Research Centre, 675 West 10th Avenue, Vancouver, British Columbia, Canada V5Z 1L3.

E-mail: ywang@bccrc.ca

Received 3 March 2005; revised 8 July 2005; accepted 11 July 2005; published online 12 September 2005

prostate cancer cells used represent the extreme end of highly advanced cancers and are not associated with original tumor stroma, now recognized as a crucial factor in the pathogenesis of cancer metastasis.^{4,5} These limitations severely restrict the predictive power of such models with regard to responses of patients' tumors to anticancer drugs in the clinic.⁶ In view of this, various groups have embarked on developing more relevant models based on xenografting of primary prostate cancer tissue in immuno-deficient mice, mainly using advanced tumors and the subcutaneous graft site.^{3,7-11} These xenograft models retain growth and histopathological features characteristic of the original cancers and have been used for rapid screening of potential therapeutics. Unfortunately, xenografting to the subcutaneous site was associated with exceedingly low tumor take rates and only successful in case of highly advanced malignancies. Furthermore, the subcutaneous compartment is a nonorthotopic graft site. It does not mimic the original tumor microenvironment, nor does it allow full expression of metastatic potential. These deficiencies reduce the usefulness of such models for studies of prostate cancer metastasis.¹²

Recently, we have developed a procedure for successfully grafting and serially transplanting primary human prostate cancer tissues in SCID mice.¹³ Using the subrenal capsule graft site, we have consistently achieved high tumor take rates (>95%). This was a consequence of utilizing: (i) SCID mouse hosts, (ii) adjustment of the hormonal status of the host, if required and (iii) meticulous surgical technique. In the present study, we found that transplants of subrenal capsule xenografts of human prostate cancer tissue, as distinct from primary tumor tissue, were able to grow in the orthotopic site (prostate) of SCID mice. The metastatic behavior of the grafts at this site led to isolation of a transplantable, metastatic tumor subline, designated PCa1-met. Orthotopic xenografts in SCID mice of the transplantable tumor and its metastatic subline led to tumors showing histopathological, tissue invasive and metastatic features, which are similar to those normally encountered in the clinic. Such xenografts could therefore be useful as orthotopic models for human metastatic prostate cancer.

Materials and methods

Materials and Animals

Chemicals, stains, solvents and solutions were obtained from Sigma-Aldrich Canada Ltd, Oakville, ON, Canada, unless otherwise indicated. Male 6- to 8-week old nonobese diabetic, severe combined immuno-deficient (NOD-SCID) mice were bred by the BC Cancer Research Centre Animal Resource Centre, BC Cancer Agency, Vancouver, Canada.

Cell Cultures

The human prostate cancer cell line, LNCaP, was obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in RPMI-1640 medium (Stem Cell Technologies, Vancouver, BC, Canada), supplemented with 10% fetal bovine serum (Gibco-BRL, Burlington, ON, Canada), penicillin (50 units/ml) and streptomycin (50 µg/ml) (Stem Cell Technologies) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Subculturing was carried out using standard techniques, including trypsinization using 0.25% trypsin/1.0 mM EDTA.

Prostate Cancer Tissue Acquisition

Prostate cancer tissue was obtained via prostatectomy from a 75-year-old male, with informed consent, at the Urology Research Unit, Carlton Centre, San Fernando, Trinidad. The patient, diagnosed with advanced prostate cancer, had not received neoadjuvant therapy prior to prostatectomy. The specimens were examined, sectioned and selected by pathologists for histological analysis and xenografting. The tumor sections selected for grafting were shipped overnight, immersed in cold Hanks' balanced salt solution supplemented with antibiotics to Vancouver, Canada.

Subrenal Capsule Grafting and Development of Transplantable Tumor Lines

Within 24 h of its arrival, a minor portion of the tumor was fixed for histological analysis. The remainder of the tumor was cut into approximately 300 pieces with each piece about 1 × 3 × 3 mm³ in size. A total of 70 NOD-SCID mice were supplemented with testosterone (25 mg) via subcutaneously implanted testosterone pellets.^{14,15} The tumor pieces were grafted to the subrenal capsule sites of the mice. Under sterile conditions, a skin incision of approximately 2 cm was made along the dorsal midline of an anesthetized mouse. With the animal lying on its side, an incision was then made in the body wall slightly shorter than the long axis of the kidney. The kidney was slipped out of the body cavity by applying pressure on the other side of the organ using forefinger and thumb. After exteriorization of the kidney, #5 fine forceps were used to gently pinch and lift the capsule from the renal parenchyma to allow a 2–4 mm incision in the capsule using fine spring-loaded scissors. A pocket between the kidney capsule and the parenchyma was then created by blunt dissection. Care was taken not to damage the parenchyma and thus prevent bleeding. The graft was transferred to the surface of the kidney using blunt-ended forceps. The cut edge of the renal capsule was lifted with fine forceps, and the graft was inserted into the pocket under the

capsule using a fire-polished glass pipette. Two or three grafts per kidney could be placed under the renal capsule. The kidney was then gently eased back into the body cavity and the body wall and skin incisions sutured. Mice were housed in groups of three in microisolators with free access to food and water and their health was monitored daily. Animal care and experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

After 60 or 90 days of growth (or earlier if required by the health status of the hosts), the animals were killed in a CO₂ chamber for necropsy. Tumors were harvested, measured, photographed and fixed for histopathological analysis. Some of the rapidly growing tumors were maintained for up to eight transplant generations by serial subrenal capsule transplantation into testosterone-supplemented male NOD-SCID mice. One of these transplantable lines, PCa1, was used for the study presented here.

Orthotopic Grafting and Isolation of a Metastatic Tumor Subline, PCa1-met

Tumor tissue of the transplantable PCa1 line was harvested from a kidney and cut into pieces of about 2 mm³. These were grafted into the anterior prostates of 12 male, testosterone-supplemented NOD-SCID mice (two per mouse). Briefly, a transverse incision was made in the lower abdomen and the bladder, seminal vesicles and prostate were partially pulled out of the abdominal cavity to expose the anterior prostate. A 2–3 mm incision was made through the capsule of the anterior prostate between the two main ducts. A 2 mm³ PCa1 tumor graft was inserted into the pocket created under the prostatic capsule. The exteriorized organs were then returned into the body cavity, and the incisions in the body wall and skin closed using a running suture of 4-0 silk. After 6 weeks of growth, the host mice were killed for gross examination of lymph nodes. Swollen lymph nodes were bisected, and one-half subjected to histological examination as frozen sections. Confirmed lymph node metastases were designated 'PCa1-met'. Pieces of fresh lymph node containing metastatic deposits were transplanted into prostates of new hosts and the emerging PCa1-met line was subsequently maintained by serial transplantation both in prostates and under renal capsules of NOD-SCID mice. The procedure for selecting PCa1-met is illustrated in Figure 1.

Development of Multiorgan Metastases

PCa1-met tissue, freshly isolated from subrenal capsule sites of NOD-SCID mice, was regrafted into anterior prostates of 47 male, testosterone-supplemented NOD-SCID mice (divided into five groups). At intervals of 2, 3, 4, 6 and 8 weeks after grafting, mice were killed and lymph nodes, lungs, livers,

kidneys, spleens and bone (femur) were fixed for histological and immunohistochemical (IHC) examination or frozen in liquid nitrogen for RT-PCR analysis.

Histological and IHC Staining

The original tumor specimen, its transplants and metastases were fixed in 10% neutral-buffered formalin and embedded in paraffin; for bone metastases, specimens were treated, after fixing, with a sterile decalcification solution (15% EDTA/0.5% paraformaldehyde in PBS, pH 8.0) for 3 weeks prior to paraffin embedding. Serial sections (5 μm thick) were cut on a microtome and mounted on glass slides. Approximately 80 sections were cut from each paraffin block. For histopathological examination, every fourth section was de-waxed in Histoclear (National Diagnostic, Atlanta, GA, USA) and hydrated in graded alcohol solutions and distilled water for H&E staining and examined under a light microscope. For IHC staining, endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in methanol for 30 min followed by washing in PBS pH 7.4. In all, 5% normal goat or donkey serum in PBS was applied to the sections for 30 min to block nonspecific sites. The sections were then incubated with primary antibodies overnight at 4°C or with control IgG from nonimmunized mice. Rabbit polyclonal anti-androgen receptor (AR) antibodies (PA1-111A) were purchased from Affinity BioReagents (Golden, CO, USA) and mouse anti-human mitochondria monoclonal antibodies (MAB-1273) from Chemicon International (Temecula, CA, USA). An anti-CK8 mouse monoclonal antibody preparation (LE41) was generously provided by Dr EB Lane, University of Dundee, UK. Polyclonal rabbit antibodies against human prostate specific antigen (PSA) and mouse anti-human Ki-67 antibodies (m7240) were purchased from Dako (Carpinteria, CA, USA); these two antibodies reacted with human, but not with mouse tissues. Purified rabbit and mouse IgGs were obtained from Zymed Corp. (So. San Francisco, CA, USA). Biotinylated anti-rabbit and anti-mouse IgGs were obtained from Amersham International (Arlington Heights, IL, USA). Biotinylated anti-goat antibodies were purchased from Sigma. Peroxidase-linked avidin/biotin complex reagents were obtained from Vector Laboratories (Burlingame, CA, USA). Following incubation with the primary antibodies, sections were washed with PBS and incubated for 30 min at room temperature with the appropriate biotinylated secondary anti-mouse immunoglobulins diluted with PBS 1:200. After incubation with the secondary antibodies, sections were washed in PBS (three 10-min washes), and then incubated with avidin-biotin complex (Vector Laboratories, Foster City, CA, USA) for 30 min at room temperature. Following a further 30 min of washing in PBS, immunoreactivity was visualized using 3,3'-diaminobenzidine tetra-

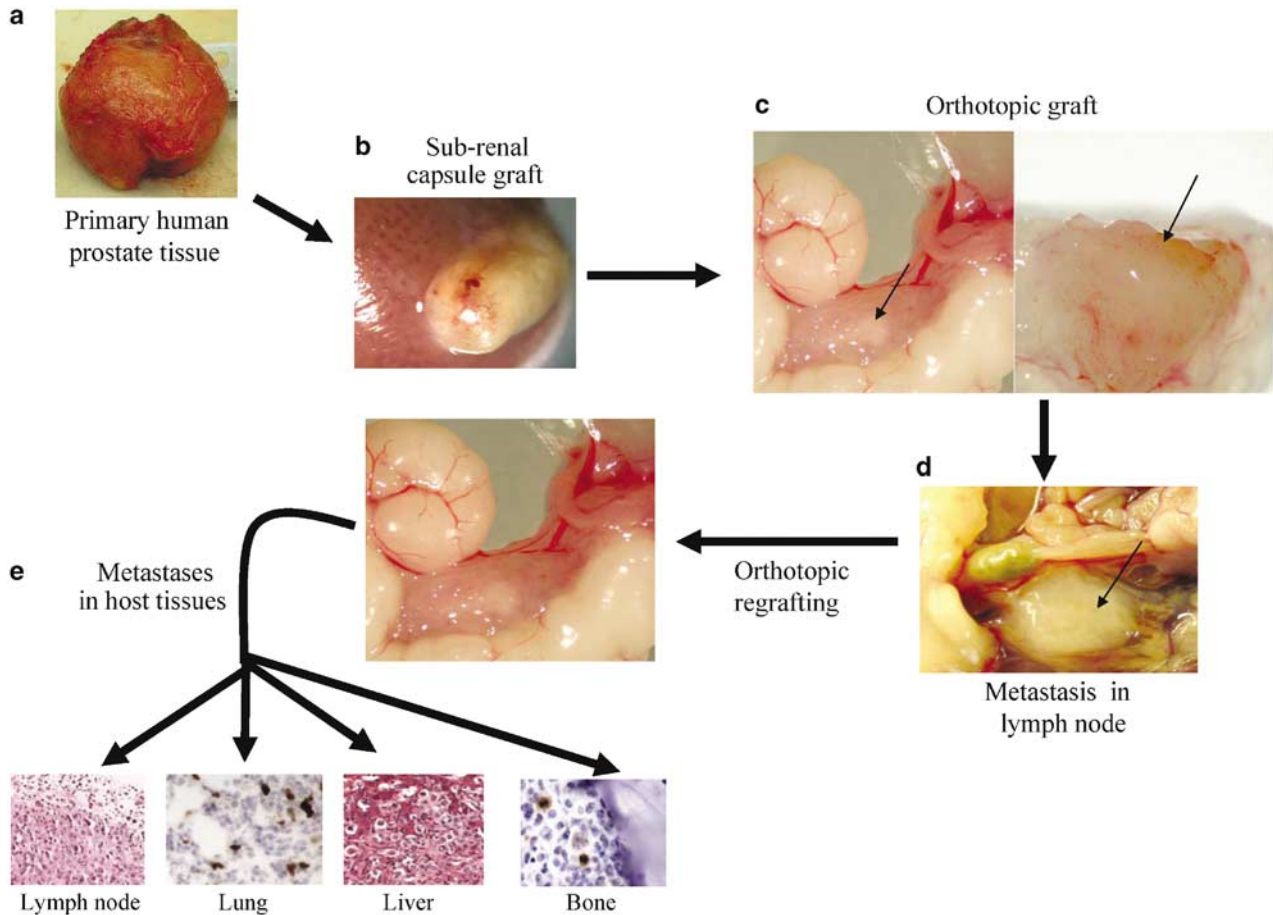


Figure 1 Establishing an orthotopic metastatic prostate cancer model. Primary human prostate cancer tissue (a) was grafted under renal capsules of testosterone-supplemented male NOD-SCID mice (b). After successful serial subrenal capsule transplantations of tumor grafts (up to 8 times), tumor tissue was regrafted into anterior prostates of NOD-SCID mice (c, arrows). These orthotopic grafts gave rise to lymph node metastases (d, arrow), which were successfully regrafted into mouse prostates, giving rise to metastases (e) in the lymph node (H&E), lung (Ki-67 staining), liver (H&E) and bone (Ki-67 staining).

hydrochloride (DAB) in PBS and 0.03% hydrogen peroxide. Sections were counterstained with hematoxylin and dehydrated in graded alcohols. Control sections were processed in parallel with mouse or rabbit nonimmune IgG (Dako) used at the same concentrations as the primary antibodies.

RT-PCR

Total RNA was isolated from tumor tissue using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A 1 μ g quantity was used for one-step RT-PCR (Invitrogen) with the following thermal cycling conditions: one cycle at 50°C for 30 min, 94°C for 2 min with an additional 29 cycles for both GAPDH and mouse β 2-microglobulin at 94°C for 15 s, 62°C for 30 s and 72°C for 1 min and a final extension at 72°C for 5 min. For the nested PSA RT-PCR, 1 μ g of total RNA was subjected to one-step RT-PCR (Invitrogen) using the external primers under the following conditions: one cycle at 50°C for 30 min, at 94°C for 2 min and 25 cycles at 94°C for 15 s, at 62°C for 30 s and 72°C for

1 min and a final extension at 72°C for 5 min. In all, 1 μ l from this PCR product was subjected to a second PCR run using the internal primers with the following conditions: 94°C for 2 min and 25 cycles at 94°C for 15 s, 62°C for 30 s and 72°C for 1 min and a final extension at 72°C for 5 min. Sequences of the primers used are as follows. External primers for PSA: sense-GAT GAC TCC AGC CAC GAC CT, antisense-CAC AGA CAC CCC ATC CTA TC; inner primers for PSA: sense-GCA AGT TCA CCC TCA GAA GG, antisense-GAT ATG TCT CCA GGC ATG GC; primers for human GAPDH: sense-CCG AGC CAC ATC GCT CAG A, antisense-CCC AGC CTT CTC CAT GGT G; primers for mouse β 2-microglobulin: sense-CAC GCC ACC CAC CGC AGA ATG GGA AGC C, antisense-CTG CGT GCA TAA ATT GTA TAG CAT ATT AG.

Western Blot Analysis

The mouse and xenograft tissues were ground up with the aid of mortar and pestle using liquid nitrogen and then homogenized in ice-cold RIPA

buffer (40 mM Tris-HCl, pH 7.0/1 mM EDTA/4% glycerol/10 mM DTT/0.2% SDS/20 mM Na molybdate/50 mM NaF/complete protease inhibitors (Roche)) using a polytron homogenizer. Protein concentrations were determined by RC DC assay (BioRad). In all, 50 μ g of protein was resolved on an 8.5% SDS-polyacrylamide gel and transferred to nitrocellulose membrane. The membrane was blocked for 1 h in 5% (w/v) nonfat dry milk in 20 mM Tris (7.6), 150 mM NaCl and 0.1% Tween-20 and then probed with anti-AR441 at 1:500 (Santa Cruz) overnight and subsequently with goat anti-mouse HRP-conjugated secondary antibody. The protein bands were detected using the enhanced chemiluminescence kit (Amersham).

Spectral Karyotyping Analysis

PCa1-met tumor tissue was harvested to produce metaphase spreads via primary cell cultures. To this end, tumor tissue fragments (about 3–5 mm in greatest dimension) were finely minced into a paste and incubated in tissue culture medium at 37°C, as described above. When cultures grew to about 40% confluency, cells were incubated with 0.1 μ g/ml colcemid (Gibco/BRL) for 30 min followed by 10% trypsin (Invitrogen) for 5–10 min. Cell swelling, cell fixation, metaphase slide preparation and pretreatment were performed as previously described.^{16,17} Spectral karyotyping (SKY) painting and detection were performed using the SKY™ kit probe cocktail according to the manufacturer's recommendations (Applied Spectral Imaging, Carlsbad, CA, USA). Pretreated slides were denatured in 70% formamide/2 \times SSC at 75°C for 1–2 min. The SKY probe was denatured for 7 min at 75°C and reannealed at 37°C for 1 h. After hybridization for 16–40 h at 37°C in a humid dark chamber, posthybridization washes and detection were performed as per the manufacturer's directions. Acquisition of hybridization signals and corresponding DAPI-stained chromosome spreads was carried out using the SD 200 bio-imaging system and software (ASI Ltd, MigdalHaemek, Israel) attached to a Zeiss Axioplan-2 Microscope (Carl Zeiss, Canada). SKY was performed using SKYview software version 1.6.2 (ASI, Ltd) as previously described and in keeping with ISCN conventions.^{16,17}

Results

Characterization of a Patient-Derived Prostate Cancer Line, PCa1

The transplantable PCa1 tumor line was derived from a subrenal capsule xenograft of primary prostate cancer tissue in a NOD-SCID mouse (see Figure 1), supplemented with testosterone to overcome xenograft atrophy due to intrinsically low levels of the hormone in SCID mice.¹³ It was

propagated by serial subrenal capsule xenografting in testosterone-supplemented male NOD-SCID mice for up to eight transplant generations. The tumor volume doubling time in such mice was approximately 7 days. Similar to the primary tumor, the PCa1 xenografts were poorly differentiated, generally lacking glandular differentiation (Figures 2a and d). The primary tumor cells expressed human AR and PSA as indicated by IHC staining using anti-human AR and PSA antibodies (Figures 2b and c) with high specificity as indicated by positive and negative controls (Figures 2h and j); likewise, the PCa1 xenografts expressed human AR and PSA, although weakly but still detectable by IHC (Figures 2e and f), thus confirming their human prostatic origin. Taken together, the data indicate that the PCa1 tumor line represents an actively growing human prostatic adenocarcinoma.

Development of a Metastatic PCa1 Subline, PCa1-met

PCa1 tissue could be grown successfully in anterior prostates of testosterone-supplemented NOD-SCID mice, in contrast to primary prostatic cancer tissue whose grafting at this site was less effective.¹³ At 6 weeks after orthotopic grafting of PCa1 tissue, lymph nodes of two out of 12 mice were found to contain metastatic foci. Metastatic cancer in one of the hosts, designated PCa1-met, was further developed by grafting metastases-containing lymph node tissue in anterior prostates and subsequent transplantation of PCa1-met tissue in prostates or under the renal capsule (see Figure 1). Figure 3 shows lymph node metastases of PCa1-met at gross (Figure 3a) and microscopic (Figure 3b) levels. The metastatic cells stained positively for human mitochondria (Figure 3c) and human Ki-67 (Figure 3d). The metastatic deposits also weakly expressed AR (Figure 3e) and PSA (Figure 3f). Detection of expression of human Ki-67, PSA and mitochondria provide evidence that the PCa1-met cells are of human prostatic origin.

Development of Multiorgan Metastases from Orthotopic PCa1-met Tissue Xenografts

Grafting of PCa1-met tissue into anterior prostates of NOD-SCID mice produced tumor take rates >95% (260 out of 270 grafts). The grafts showed poorly differentiated features and local invasion of host's prostate tissue (see Figure 4a). An adjacent section (Figure 4b) stained positively with anti-human mitochondria antibody. Furthermore, the orthotopic grafts weakly expressed AR as observed with PCa1 cells (data not shown). After approximately 2 weeks of grafting the mice developed multiorgan metastases. As shown in Table 1, the animals developed metastases in lymph nodes, lung, liver, kidney, spleen and bone. The histology (H&E staining) of the metastases in the lung (Figure 4c), liver (Figure

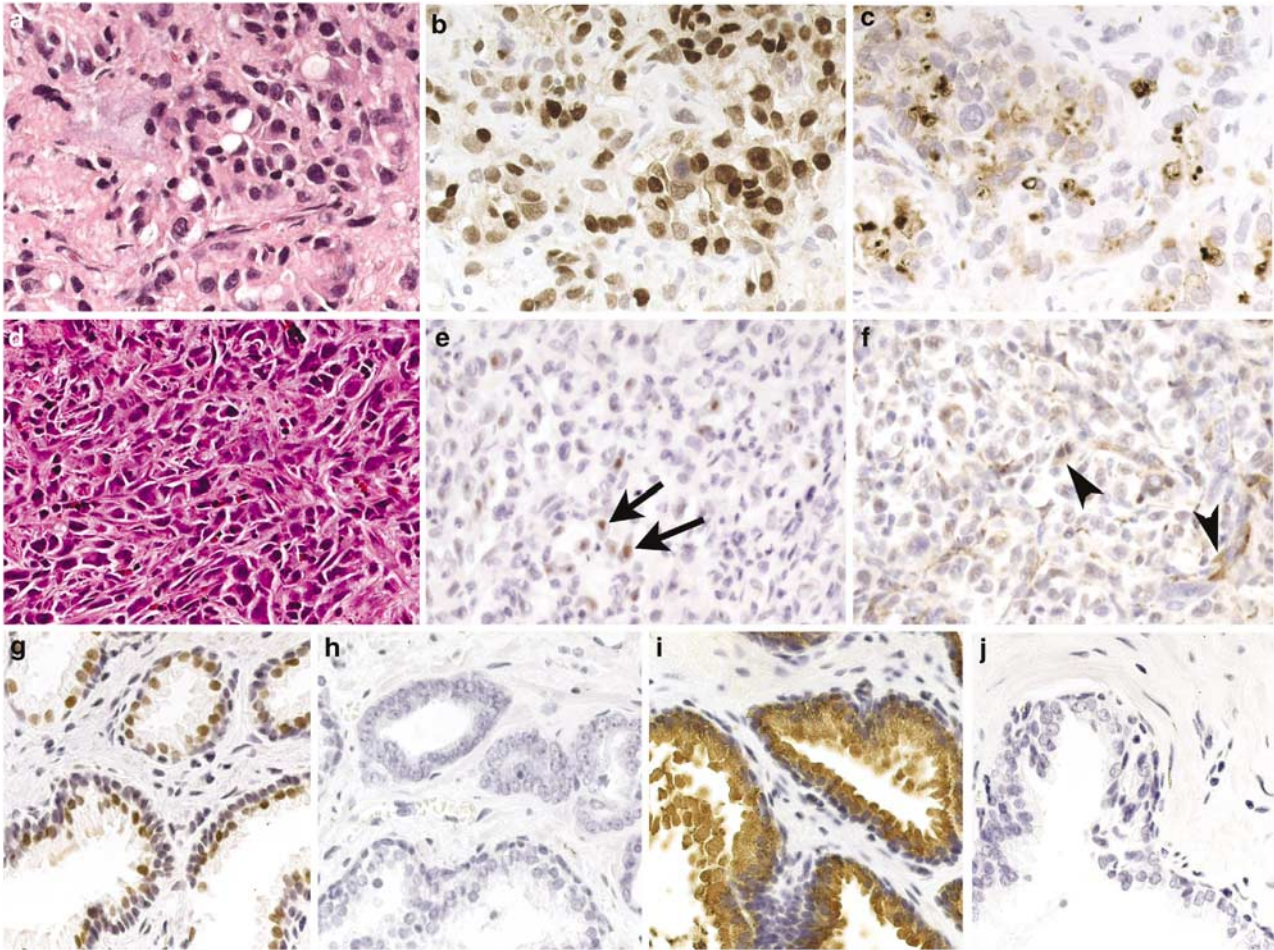


Figure 2 Sections of human primary prostate cancer before grafting (a–c) and of the transplantable PCa1 tumor line developed by serial subrenal capsule transplantation (d–f). The primary cancer is poorly differentiated and lacks glandular differentiation (a, H&E staining), but is positive for AR (b) and PSA (c). The transplantable PCa1 tumor line retains poor differentiation and lack of glandular structure (d), and is weakly positive for ARs (e, arrows) and PSA (f, arrowheads), indicating that it was derived from human prostate cancer. All IHC staining experiments were carried out using both positive (g, AR; i, PSA) and negative (h, AR; j, PSA) controls using benign human prostate tissue.

5a), kidney (data not shown) and spleen (Figure 6a) was similar to that of the original PCa1-met (Figure 3). Metastases in the lung (Figure 4d), liver (Figure 5b), kidney (data not shown) and spleen (Figure 6b) were positively stained with anti-human mitochondria antibodies. Similarly, human-specific Ki-67 staining was detected in metastases to lung (Figure 4e), liver (Figure 5c), kidney (data not shown) and spleen (Figure 6c). This demonstrates that the metastases were highly proliferative. IHC analyses confirmed weak expression of AR in metastases in the lung (Figure 4f), liver (Figure 5d), kidney (data not shown) and spleen (Figure 6d); in contrast, AR expression was negative in host tissues.

Using light microscopy of sections of host's femur, micrometastases of human origin in the marrow were detected via staining with specific anti-human mitochondria antibody (Figure 7a, arrows). Gross bone metastases, however, were not observed in any of the engrafted animals. The human origin of the

micro-metastases was also confirmed by RT-PCR for human GAPDH (data not shown). Supporting evidence for their human prostatic origin was obtained via nested RT-PCR showing that human PSA mRNA was associated with PCa1-met tissue as well as with bone marrow (Figure 7d). Similarly, human AR protein was found to be associated with PCa1-met tissue (Figure 7e). At high magnification, H&E-staining (Figure 7b) shows that the metastatic human prostate cancer cells in a bone lesion had histologic and cytologic features of the original tumor cells, including a solid growth pattern, moderately pale to clear cytoplasm, oval nuclei and nucleoli and, importantly, produced osteolytic lesions as demonstrated by replacement of marrow and resorption of bony trabeculae (Figure 7b). An adjacent section, stained with anti-human mitochondria antibody (Figure 7c), shows cytoplasmic immunoreactivity within metastatic tumor cells whereas the remaining bone tissue is negative.

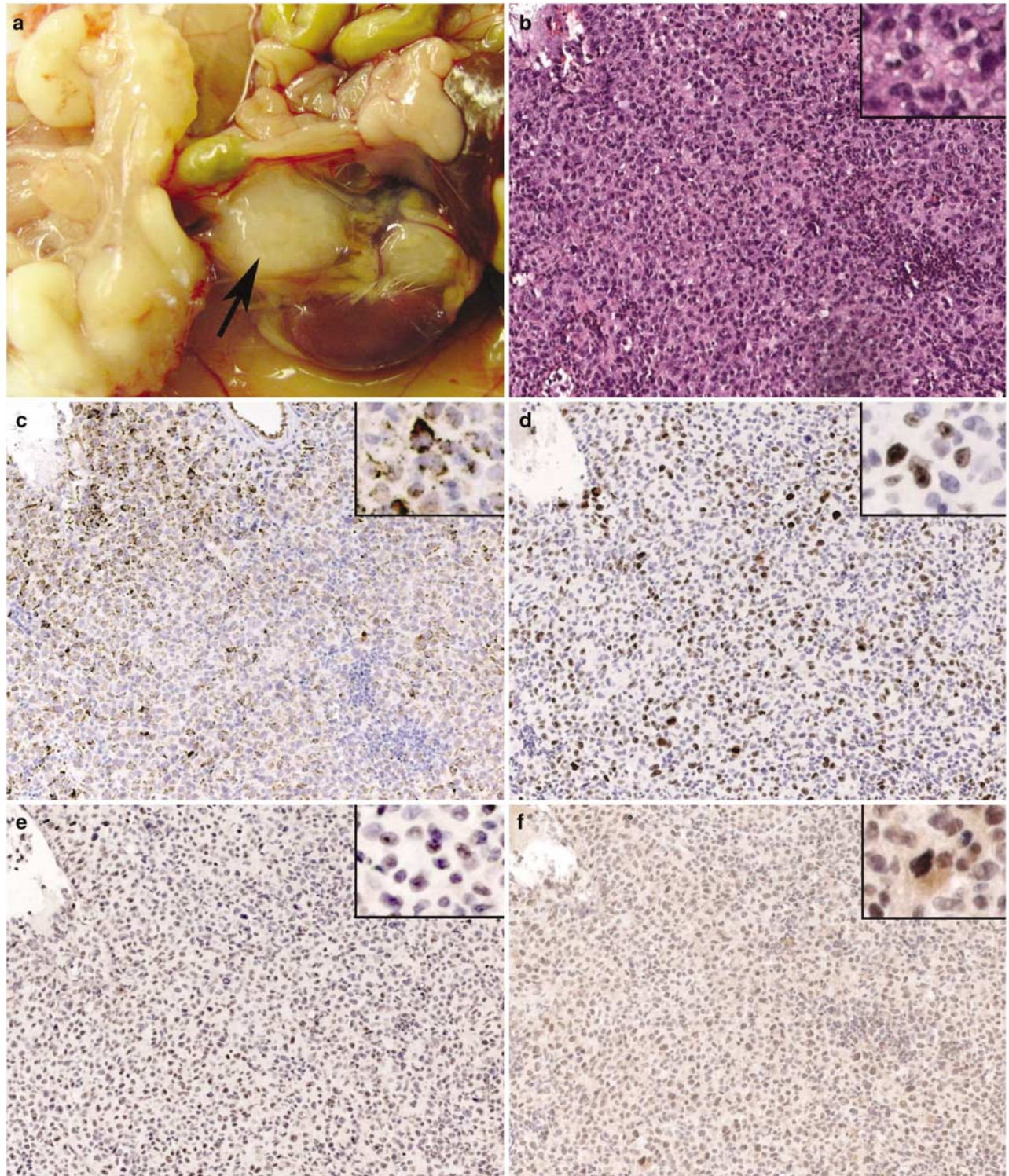


Figure 3 Lymph node metastases from orthotopic xenografts of the PCa1-met prostate cancer tissue subline. Gross metastasis (a, arrow) and microscopic sections of PCa1-met lymph node metastases (b, H&E), showing histological similarity with the parental subrenal capsule xenografts (see Figure 2). Immunostaining is positive for human mitochondria (c), Ki-67 (d), AR (e) and PSA (f), indicating that the lymph node metastases are derived from human prostate cancer.

SKY Analysis of PCa1-met

Primary PCa1-met tissue cultures were harvested after 5 days of incubation and subsequent 30-min treat-

ment with colcemid. The number of metaphases obtained was very low (<1% metaphase count), but sufficient to obtain a consensus SKY karyotype. PCa1-met cells were diploid with a modal chromosome

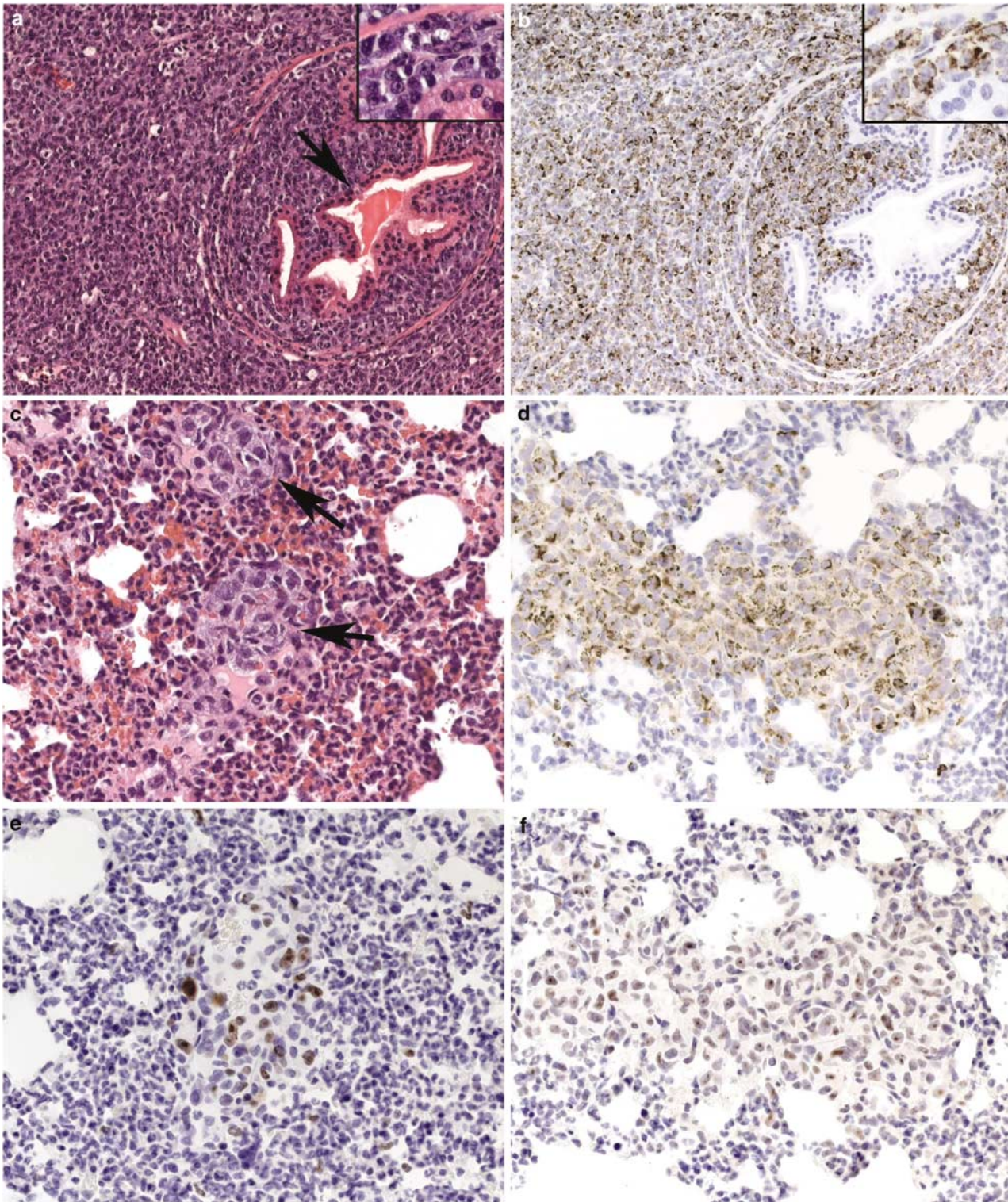


Figure 4 Sections of an orthotopic graft of PCa1-met human prostate cancer tissue and its lung metastases. Tumor cells have grown around a mouse host prostatic duct (**a**, H&E, arrow), staining positively with anti-human mitochondria antibody, while the host's prostatic ductal epithelial cells are negative (**b**). Two metastatic foci are present in the lung parenchyma (**c**, H&E, arrows). The tumor cells stain positively with anti-human mitochondria (**d**), anti-Ki-67 (**e**) and anti-AR (**f**) antibodies.

number of 45 and loss of the intact Y chromosome. Sporadic (ie nonclonal, nonrecurring) chromosomal aberrations were infrequent and the number of clonal abnormalities was small. Figure 8 shows the

PCa1-met karyotype which is identical to that of PCa1 (not shown). The most frequent clonal changes observed consisted of three unbalanced translocations involving six chromosomes (#4, 6, 12, 13, 22 and Y).

Discussion

Therapy-resistant metastasis is the leading cause of human prostate cancer death.¹ Currently, there are no effective therapies for metastatic prostate cancer, and this deficiency is in part a reflection of a lack of understanding of the mechanisms involved in the progression of the disease.² Research in this area has been hampered by a lack of clinically relevant, experimental models.³ For example, certain models

Table 1 Incidence of prostate cancer metastases in host tissues (%)

Weeks after grafting (# of mice)	Lymph nodes	Lung	Liver	Spleen	Kidney	Bone
2 (10)	80	30	0	0	0	0
3 (10)	100	100	30	30	10	0
4 (10)	100	100	80	90	40	0
6 (10)	100	100	90	90	100	0
8 (7)	100	100	100	100	100	43

are based on injection of highly anaplastic human prostate cancer cells into the bloodstream or into target organs (eg, tibia) of immuno-deficient mice and produce 'experimental metastatic foci'.¹⁸ Such foci are not a consequence of the normal metastatic behavior of cancer cells (intravasation, migration, extravasation). Instead, they rather reflect the ability of the injected cells to proliferate in certain distant locations. In contrast, models based on implantation of prostate cancer tissue, as distinct from cells, into prostates of experimental animals appear clinically more appropriate, since an implantation site is used that is more representative of the original tumor environment.¹⁹

There is accumulating evidence that metastatic behavior of a neoplasm is governed not only by intrinsic tumor factors, but also to a large extent by multiple interactions between cancer cells and their microenvironment promoting metastasis of discrete tumor subpopulations.⁴ Tumor-associated macrophages, for example, can induce matrix breakdown, tumor cell motility and angiogenesis.^{20,21} In establishing a model that represents a patient's tumor, it

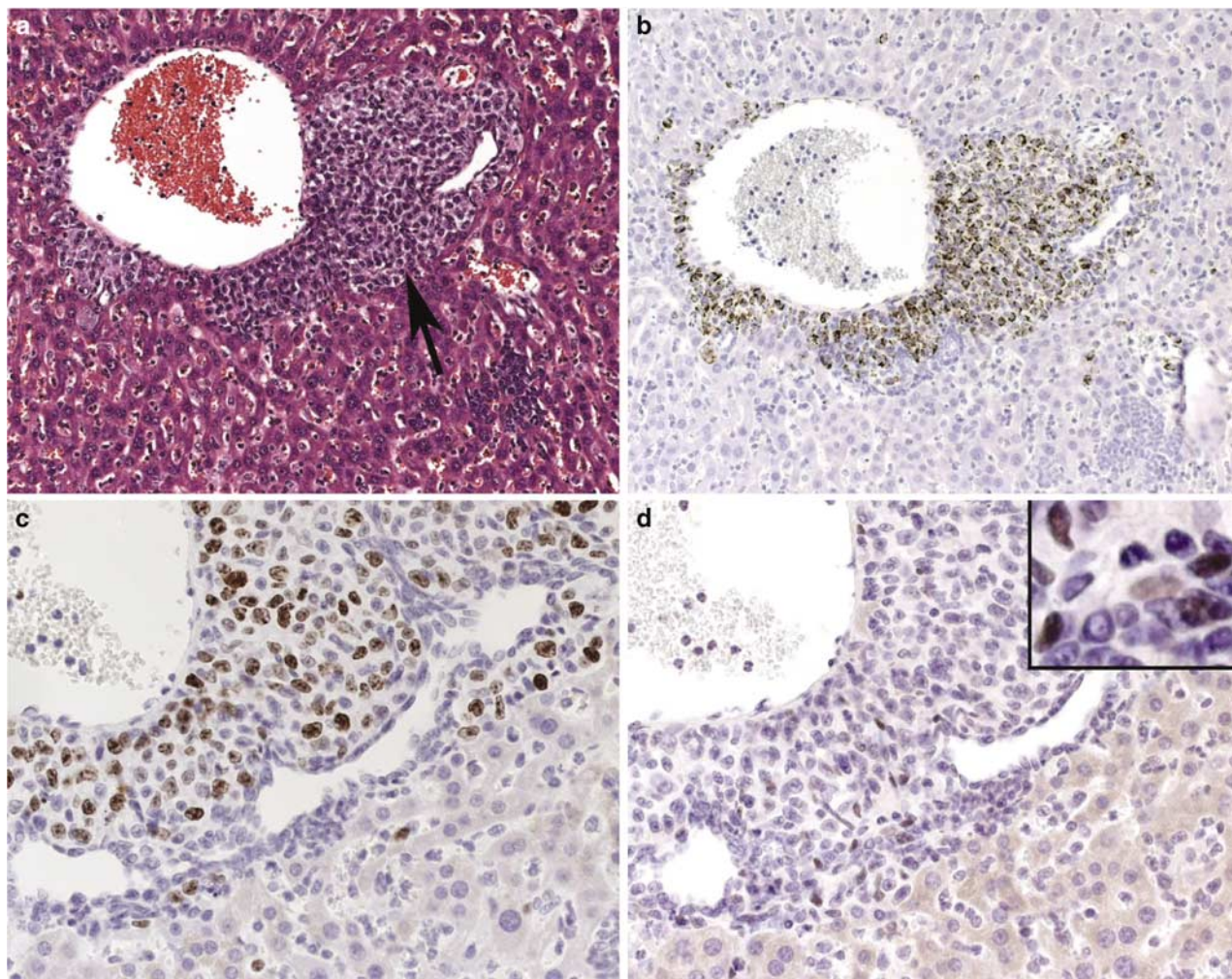


Figure 5 Sections of liver metastases from orthotopically engrafted PCa1-met human prostate cancer tissue. Tumor cells are present in the liver parenchyma (a, H&E, arrow), staining positively with anti-human mitochondria (b), anti-Ki-67 (c) and anti-AR (d) antibodies.

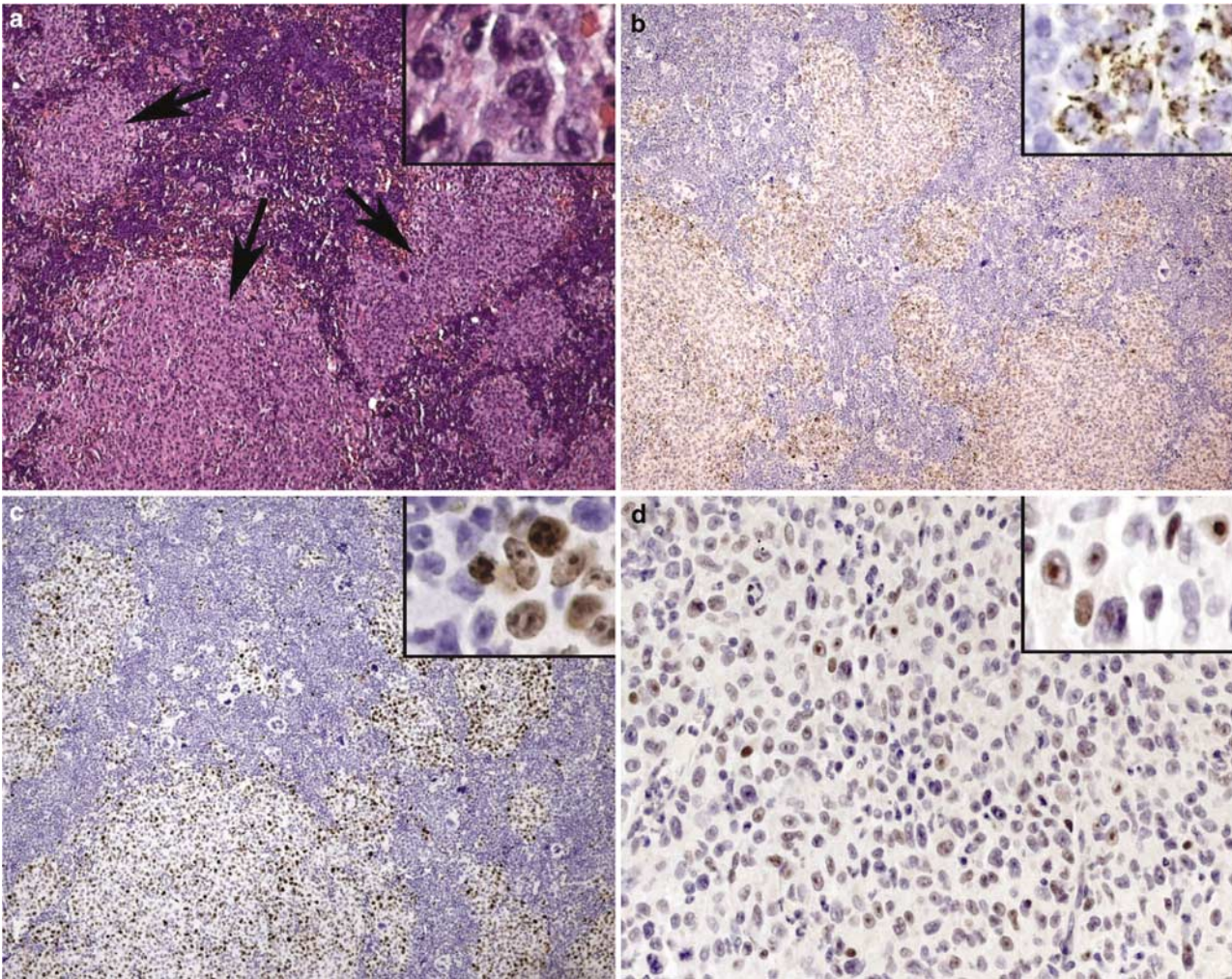


Figure 6 Sections of spleen metastases from orthotopically engrafted PCa1-met human prostate cancer tissue. Foci of tumor cells are present in splenic parenchyma (a, H&E, arrows), staining positively with anti-human mitochondria (b), anti-Ki-67 (c) and anti-AR (d) antibodies.

is therefore essential that the grafted specimen contains all of the crucial components of the original tumor and that these are retained in the grafts. In this regard, it is notable that the initial subrenal capsule xenografts of prostate cancer tissue indeed contained human stroma which, however, was gradually replaced by mouse stroma.²²

Primary prostatic neoplasms are thought to consist of a variety of carcinoma subpopulations showing differences in invasive and metastatic properties.⁷ The present study shows that prostate cancer tissue from a patient can be readily grown in prostates of NOD-SCID mice following its establishment as subrenal capsule xenografts. It appears that the high tumor take rate achievable with the subrenal capsule grafting technique, for both low and higher grade cancers, minimizes the chance of losing subpopulations of the original prostatectomy specimen.¹³ Potential loss of such subpopulations appears much greater when other grafting sites with much lower engraftment rates are used (eg the subcutaneous compartment). The PCa1 line,

developed in this study, represents an actively growing human prostatic adenocarcinoma line with poorly differentiated features and general lack of glandular differentiation (Figure 2). PCa1-met, the metastatic subline derived from lymph node metastases of the PCa1 line, resembles its parental line with regard to poorly differentiated features, as well as its human origin and expression of AR and PSA (Figures 3 and 4). The metastases in the mice receiving orthotopic transplantation of PCa1-met tissue were located in lymph nodes, lung, liver, kidney, spleen and bone (Figures 3–7), which are common metastatic sites in prostate cancer patients. The finding in mouse bone of orthotopically grafted cancer cells of prostatic origin and exhibiting osteolytic action is particularly interesting, since this phenomenon is not observed with other currently available metastatic prostate cancer models.²³ It appears therefore that a subline such as the PCa1-met, produced by subrenal capsule and subsequent orthotopic xenografting, is highly suitable for use in NOD-SCID mice as a model for

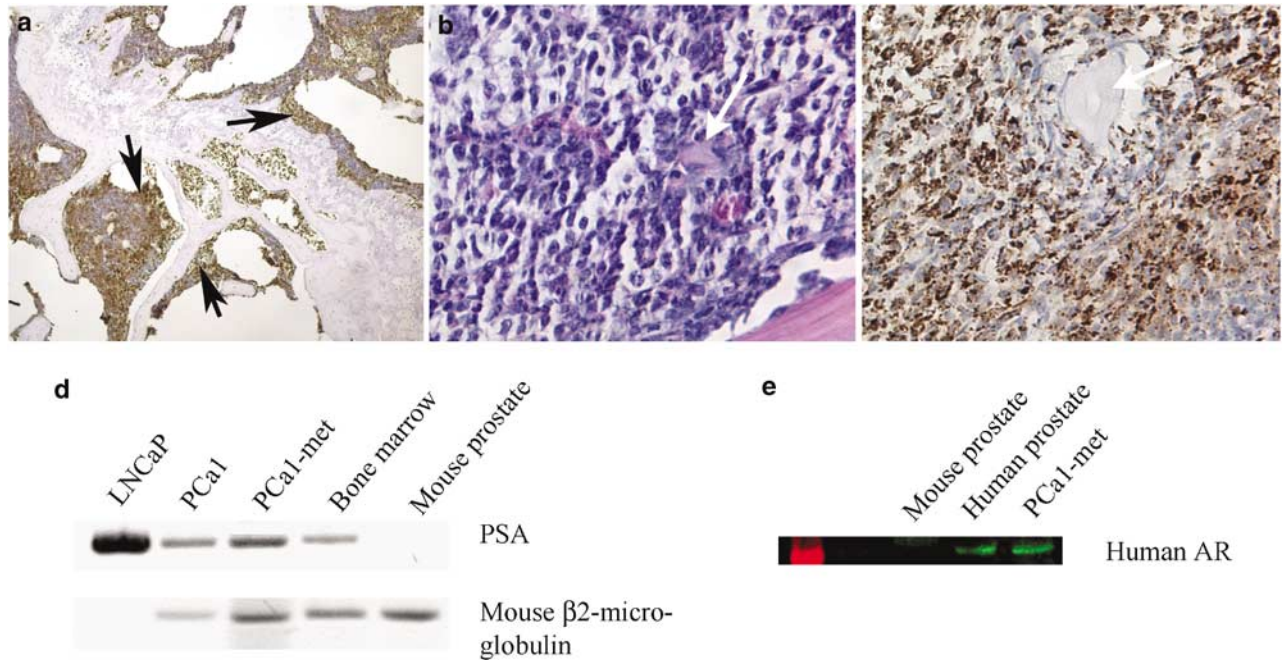


Figure 7 Sections of bone marrow containing metastases from orthotopically engrafted PCa1-met human prostate cancer tissue. Low-power photomicrograph (produced by IHC staining using anti-human mitochondria) showing replacement of marrow elements by metastatic carcinoma (a, arrows) with thinning of bony trabeculae resulting in osteolytic appearance. Higher power photomicrographs showing sheets of tumor cells and a single thinned trabecula (arrows) (b, H&E staining; c, staining with anti-human mitochondria antibodies). (d) RT-PCR products for PSA (human) and $\beta 2$ -microglobulin (mouse; control) using total RNA harvested from tissues. (e) Western blot of human AR from various tissues.

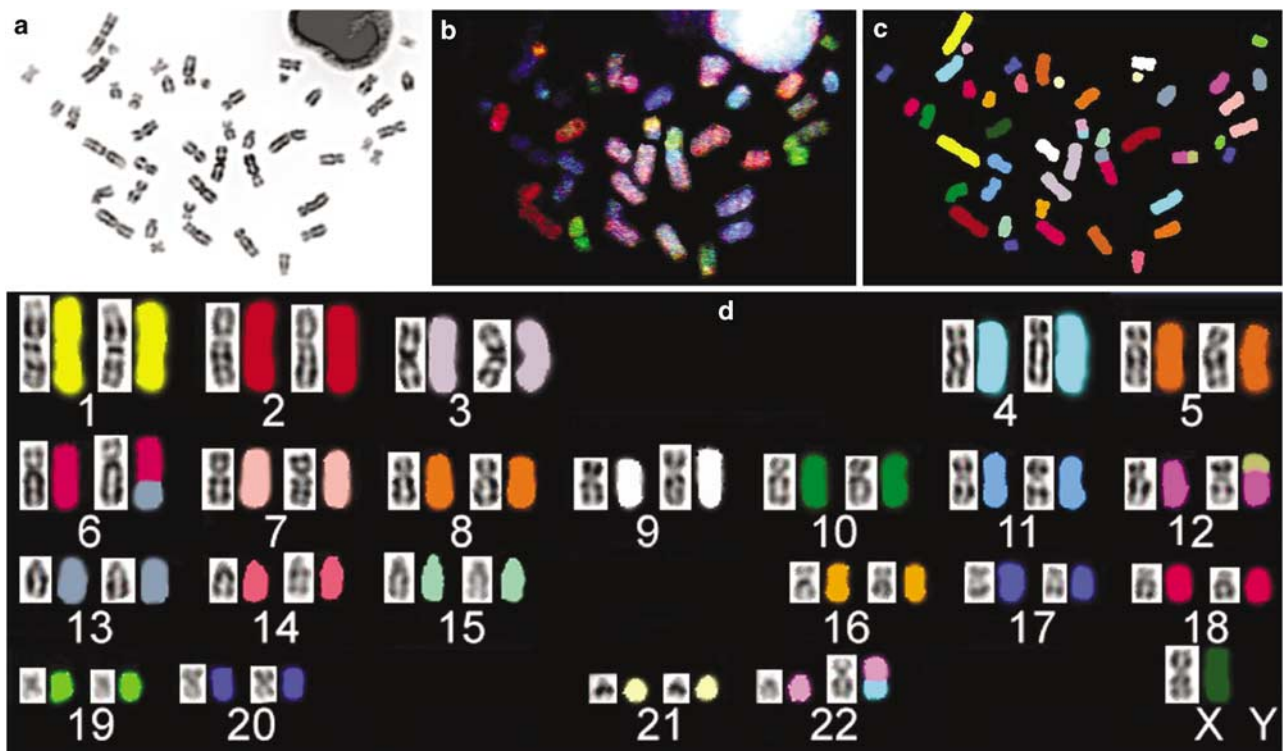


Figure 8 Spectral karyotype composite of the PCa1-met human prostate cancer line. DAPI-banded preparation of metaphase chromosomes from a cell following short-term culture (a), hybridized to SKY paints (b) and after pseudocolor classification (c). Resultant karyotype table is presented in (d). A SKY profile of PCa1-met: 45, X,-Y,der(6)t(6;13)(q24;q22), der(12)t(Y;12)(q11;p11), der(22)t(4;22)(?;p11).

metastatic prostate cancer. It may be noted that the PCa1-met line was found to be androgen-independent, growing readily in mice following androgen ablation (unpublished observation).

SKY analyses of three widely used prostate cancer cell lines (LNCaP, DU-145, PC-3) have demonstrated aneuploid karyotypes with many chromosomal alterations including complex chromosomal rearrangements and a high degree of karyotypic instability. No common chromosomal rearrangement or translocation breakpoint was identified.^{24,25} In contrast to these highly anaplastic cell lines, early-stage prostatic tumors are typically diploid with some propensity to progress to aneuploidy and karyotypic complexity in advanced stages.²⁶ The karyotype of PCa1-met is comparable to the latter, with surprisingly few chromosomal alterations produced by as few as six breakpoints involving chromosomes 4, 6, 12, 13, 22 and Y. Common involvement of chromosome 8, typically a loss of 8p with gain of 8q, reported previously,²⁷ was not seen in the present study using SKY. However, smaller but significant chromosomal alterations, such as small interstitial deletions, insertions/duplications and inversions cannot be revealed by whole-chromosome painting and would require higher-resolution techniques such as locus-specific-FISH, Mband FISH or array CGH. Loss of the Y chromosome is frequently associated with aging. However, it has also been suggested that loss of certain Y chromosome specific genes may play a role in the pathogenesis of prostate cancer.²⁸ The finding of simple and identical karyotypes in PCa1 (data not shown) and PCa1-met, its metastatic subline, suggests that significant chromosome and karyotypic instability are not characteristics of these tumor lines, in contrast to commonly used prostate cancer cell lines such as DU-145.²⁹ It is also likely that conservation of such few chromosomal changes is of biological significance, by offering selective advantage to the tumor cells and maintenance of malignant and/or metastatic properties.

In conclusion, the orthotopic prostate cancer metastasis model developed in this study should be useful for investigations of mechanisms underlying prostate cancer metastasis and therapeutic applications. The methodology used to develop this model may also be suitable for developing orthotopic metastatic cancer models of other types of human cancer. Thus, the first step of the methodology, that is, subrenal capsule grafting, has been successful for a variety of primary human cancer tissues, including cancers of the ovary,³⁰ kidney,²² lung, pancreas and lymphoid tissue (unpublished observations).

Acknowledgements

We thank Rebecca Wu and Lily Wei for excellent technical assistance. Paula Marrano is thanked for technical expertise and help in SKY analyses. This

study was supported in part by grants awarded to YZW by NCI Canada (#014053), the US Army Department of Defense, USAMRMC W81XWH-04-1-0290, the Prostate Cancer Foundation of Canada and to GRC by the National Cancer Institute (# CA89520). J-C Cutz was a CIHR fellow in Molecular Oncologic Pathology.

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