Establishing Prostate Cancer Patient Derived Xenografts: Lessons Learned From Older Studies

Pamela J. Russell,[1]* Peter Russell,[2,3] Christina Rudduck,[4] Brian W-C Tse,[1] Elizabeth D. Williams,[1] and Derek Raghavan[5]

[1]*Australian Prostate Cancer Research Centre - Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Queensland 4102, Australia*
[2]*GynaePath, Douglass Hanly Moir Pathology, Macquarie Park, New South Wales, Australia*
[3]*Department of Obstetrics Gynaecology and Neonatology, University of Sydney, Sydney, New South Wales, Australia*
[4]*Cytogenetics Department, The Children's Hospital, Sydney, New South Wales, Australia*
[5]*Levine Cancer Institute, Charlotte, North Carolina*

Background. Understanding the progression of prostate cancer to androgen-independence/castrate resistance and development of preclinical testing models are important for developing new prostate cancer therapies. This report describes studies performed 30 years ago, which demonstrate utility and shortfalls of xenografting to preclinical modeling.
Methods. We subcutaneously implanted male nude mice with small prostate cancer fragments from transurethral resection of the prostate (TURP) from 29 patients. Successful xenografts were passaged into new host mice. They were characterized using histology, immunohistochemistry for marker expression, flow cytometry for ploidy status, and in some cases by electron microscopy and response to testosterone. Two xenografts were karyotyped by G-banding.
Results. Tissues from 3/29 donors (10%) gave rise to xenografts that were successfully serially passaged in vivo. Two, (UCRU-PR-1, which subsequently was replaced by a mouse fibrosarcoma, and UCRU-PR-2, which combined epithelial and neuroendocrine features) have been described. UCRU-PR-4 line was a poorly differentiated prostatic adenocarcinoma derived from a patient who had undergone estrogen therapy and bilateral castration after his cancer relapsed. Histologically, this comprised diffusely infiltrating small acinar cell carcinoma with more solid aggregates of poorly differentiated adenocarcinoma. The xenografted line showed histology consistent with a poorly differentiated adenocarcinoma and stained positively for prostatic acid phosphatase (PAcP), epithelial membrane antigen (EMA) and the cytokeratin cocktail, CAM5.2, with weak staining for prostate specific antigen (PSA). The line failed to grow in female nude mice. Castration of three male nude mice after xenograft establishment resulted in cessation of growth in one, growth regression in another and transient growth in another, suggesting that some cells had retained androgen sensitivity. The karyotype (from passage 1) was 43–46, XY, dic(1;12)(p11;p11), der(3)t(3:?5)(q13;q13), -5, inv(7)(p15q35) x2, +add(7)(p13), add(8)(p22), add(11)(p14), add(13)(p11), add(20)(p12), -22, +r4[cp8].
Conclusions. Xenografts provide a clinically relevant model of prostate cancer, although establishing serially transplantable prostate cancer patient derived xenografts is challenging

*Correspondence to: Pamela J. Russell, Australian Prostate Cancer Research Centre - Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Queensland, 4102, Australia.
E-mail: pamela.russell@qut.edu.au
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and requires rigorous characterization and high quality starting material. Xenografting from advanced prostate cancer is more likely to succeed, as xenografting from well differentiated, localized disease has not been achieved in our experience. Strong translational correlations can be demonstrated between the clinical disease state and the xenograft model. *Prostate 75: 628–636, 2015.*

KEY WORDS: **prostate cancer; xenograft; cancer model; animal model**

INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and the sixth leading cause of death from cancer in men worldwide [1], and remains a significant international management challenge despite progress in our understanding of its biology and growth regulation. The normal development, growth and function of the prostate and most of its malignancies are under androgen regulation [2]. However, while an initial response to androgen ablation is seen in up to 80% of cases, an androgen independent form of the disease often develops and only 10–20% of patients presenting with metastases are alive after 5 years [3,4]. With the evolution of our understanding of the genome and the availability of GWS and related technological advances, broader application of preclinical models, using them as tissue amplifiers, may allow us to focus our clinical research more effectively.

The work described here was performed in the 1980 s, in the Urological Cancer Research Unit and Kanematsu Laboratories, Royal Prince Alfred Hospital, Sydney and Department of Surgery, University of Sydney. We established three new xenograft lines of human prostatic adenocarcinoma, one of which, UCRU-PR-2, appeared to show a transition from adenocarcinoma to neuroendocrine cancer, whilst UCRU-PR-4, which was derived from a CRPC contained some populations of cells that were androgen sensitive. This report describes applications to translational research in PCa using these xenografts. Also of relevance, it is noteworthy that we were able to apply a series of bladder cancer xenografts to the characterization of this disease [5–12], and were able to demonstrate the existence of a solid tumor stem cell [6,7], similarities between the human disease and xenografts [5,8], explore gene expression [9], and model the utility of novel therapies [5,10,11], further supporting the usefulness and relevance of this approach. Although our large survey of correlations between large groups of patients and banks of xenografts showed only limited clinical and translational correlations [12], focused tumor-specific studies, with well-defined questions and parameters of outcome can be used effectively to model the clinical scenario [13].

MATERIALS AND METHODS

Mice

Male BALB/c nu/nu ("nude") mice aged around 6 weeks were obtained from the Australian Atomic Energy Commission, Lucas Heights, New South Wales, Australia, and were housed in standard cages fitted with filter tops and handled in laminar flow hoods. In addition to these standard precautions, other animal species were not introduced into our laboratory, further reducing the potential for infection and cross-contamination. They were fed irradiated standard mouse diet and sterile acidified water ad libitum. The details of our methodology have been reported in full [14–17].

Prostate Cancer Xenografts

All studies were performed in accordance with guidelines of the Animal Ethics Committee of The University of Sydney. Transurethral resections from 29 patients with primary PCa were transported immediately from the operating theatre in sterile PBS and were minced. Fragments of biopsy no greater than $1\,mm^3$ were implanted subcutaneously (s.c.) bilaterally through small incisions above the scapula in male mice under general anaesthesia. When tumor nodules reached 5–10 mm in diameter they were removed from host mice and $2\,mm^3$ fragments were serially transplanted after dissection to remove stromal capsules and necrotic tissue. These were further passaged s.c., and in some cases under the renal capsule. Tumors were measured twice weekly, and tumor volumes were calculated from two tumor diameters (d) at right angles according to the following formula:

$V = \pi/6(d_1.d_2)^{3/2}$ (volume of an ellipse) as previously described [5]. Mean doubling times were calculated from a semi-logarithmic plot of volume versus time.

Testosterone Implants

To facilitate growth, implants of testosterone (4 Androsten 17β-01–3-one, Sigma Chemical Co., St. Louis, MO) were prepared [18] and 1 cm implants

were inserted intraperitoneally. Plasma testosterone levels in tumor bearing mice with implants were monitored by radioimmunoassay by Dr. Chris Howe, Department of Endocrinology, Royal Prince Alfred Hospital [19].

Light Microscopy

Specimens for light microscopy from the original tumors and xenograft passages were fixed in buffered 10% formalin and embedded in paraffin following routine processing. Tissue sections (5 μm) were stained with hematoxylin and eosin or used for immunohistochemistry.

Tumour Marker Staining (Immunohistochemistry)

Immunoperoxidase staining for epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), prostate specific antigen (PSA), prostatic acid phosphatase (PAcP), neurone specific enolase (NSE) and cytokeratins 7 and 8 (CAM 5.2) were carried out using the unlabelled antibody enzyme method (peroxidase antiperoxidase) on dewaxed paraffin sections [20]. Antibodies to NSE, PSA and CAM 5.2 were obtained from Dako Corp., Santa Barbara, CA, to CEA from Dakopatts a/s, Copenhagen, Denmark, to EMA from Sera-Lab, CSL, Australia, to PAcP from Pel-Freez, Biologicals, Rogers, AR and mouse IgG anti-vimentin, used at a dilution between 1/10 and 1/40 was from Amersham. Incubation in optimally diluted primary antisera was followed by sequential application of swine anti-rabbit immunoglobulin and PAP (Dakopatts, Copenhagen). Use of goat anti-EMA was facilitated by the addition of rabbit anti-goat immunoglobulin prior to the application of secondary antibody. Peroxidase activity was revealed by using diaminobenzidine (DAB, Sigma) and counterstained with Harris hematoxylin. CEA, EMA and keratin antisera were preabsorbed with acetone-dried powdered human spleen. Prior to keratin staining, sections were incubated with 0.1% Pronase E for 20 min at 37°C. Sections producing negative results after routine staining were retested after pronase treatment to optimize staining conditions. Appropriate positive controls were performed for each antibody, and negative controls included the omission of the primary antibody or incubation with an irrelevant first antibody.

Flow Cytometry

The cellular DNA content of specimens of the original tissue and of xenografts was measured by flow cytometry. Tissue specimens were mechanically disaggregated in a staining solution [21] containing propidium iodide (Calbiochem-Behring Corp., San Diego CA) at a final concentration of 50 μg/mL in 5% v/v Triton X-100 (Packard Instrument Co., Downers Grove, IL) with RNAase 1 mg/mL (Calbiochem-Behring Corp.) in 0.9% NaCl. Freshly prepared and washed chicken erythrocytes (3×10^5/mL) were added as an internal DNA standard. The cell suspension was then filtered through a 95 μm nominal pore size nylon mesh (Henry Simon, Sydney, Australia). Flow cytometry of total DNA content per cell was carried out using a Cytofluorograf System 50H cell sorter (Ortho Instruments, Westwood, MA) with an argon iron laser excitation source at 488 nm. A minimum of 5×10^4 cells was counted and the resultant frequency distribution histograms of DNA content were analysed [22]. The number of cells in the DNA S phase of the cell cycle could only be determined in specimens which did not have aneuploid cells present. The DNA content of aneuploid populations was expressed as a proportional increase in DNA over the diploid (2N) level. Where insufficient fresh material was available for analysis, flow cytometry measurements were carried out on nuclei from paraffin embedded material as described elsewhere [23].

Cytogenetic Analysis

Cells were cultured in liquid suspension in siliconized McCartney bottles at 1×10^6/mL in RPMI 1640 with 10% fetal bovine serum and 20 mM 4-(2-hydroxyethyl-1-piperazineethanesulfonic acid buffer) for 72 hr. Cells were treated with hypotonic potassium chloride for 20 min and fixed with Carnoy's fixative [24]. Metaphase spreads were G-banded by a modified method of Seabright [25] and cytogenetic analysis was performed according to the Paris convention [26].

RESULTS

Growth of Xenografts

Three xenografts were established from 29 implanted specimens (10%), and initially demonstrated the histological and functional traits of the tumors of origin, notwithstanding the maintenance of significant morphological and functional heterogeneity.

Fragments obtained from 29 patients undergoing transurethral resection of the prostate (TURP) were assessed for ploidy changes by flow cytometry. All of the tumors examined contained some aneuploid or tetraploid populations. Fragments from each specimen were also implanted s.c. in male BALB/c nu/nu ("nude") mice. Tumors grew after a prolonged latency period of 8–12 months in 5/29 cases, but in only three

(UCRU-PR-1, -2 and -4) were xenografts able to be further passaged. The characteristics of the human tumors from which these xenograft lines were established are shown in Table I.

UCRU-PR-1

The human prostatic adenocarcinoma (which was positive for PAcP, PSA, EMA and negative for CEA, β-subunit of human chorionic gonadotrophin, calcitonin, serotonin, and α-feto protein) had been xenografted in nude mice, and undergone two serial passages in vivo before being introduced to tissue culture following dissociation of the cells using collagenase. When the cultured cells were implanted s.c. (2×10^5 cells) in mice, a spindle cell fibrosarcoma of mouse origin which was a transformed aneuploid mouse cell line, grew [27]. This transformed line was tumorigenic both in BALB/c nu/nu (nude) mice and in heterozygous nu/+ mice, maintained the morphology of a spindle cell sarcoma and contained both diploid and aneuploid (2.58N) peaks. The cell line did not express human isozymes or human histocompatibility antigens, nor were human chromosomes present. Human DNA sequences were not detectable by human "Alu repeat" sequence element probing. The line contained retroviral long terminal repeat sequences but there was no evidence of proviral activation as shown by assaying for viral enzyme reverse transcriptase in cellular conditioned medium. One potential explanation of these findings is that tumor cells may cause transformation of neighbouring stromal cells, that this transformation may proceed in the absence of DNA transfer or activation of endogenous proviruses, and that the means of the observed transformation may involve humoral factors elaborated by the tumor cells [27].

UCRU-PR-2

The xenograft line, UCRU-PR-2, established from a primary human poorly differentiated prostatic carcinoma has been previously characterised [14,15]. It was maintained as a stable xenograft line in nude mice and showed the presence of undifferentiated carcinoma with epithelial (EMA and CEA positive) staining, but did not express androgen or estrogen receptors, PCaP, PSA or cytokeratin. In addition, the line expressed neuroendocrine characteristics, being NSE positive and containing neuro-secretory granules ultrastructurally. UCRU-PR-2 cells also synthesized and secreted ACTH, β-endorphin and somatostatin in vivo and ACTH and β-endorphin in vitro, suggesting that the gene for pro-opiomelanocortin was expressed and that processing of the molecule occurred [15].

Implantation of the tumor in different anatomical locations was performed to examine the effects of local micro-environmental factors on tumor growth and behaviour and to attempt to discern whether the existence of both epithelial and neuroendocrine characteristics were due to the co-existence of two types of tumor cells or, potentially, a pluripotent stem cell capable of giving rise to both populations [16], analogous to our observation in bladder cancer [6,7]. We were unable to clone the cells in vitro in order to use cloned populations for these studies. All tumors that grew were small-cell carcinomas. Tumor fragments implanted within muscle and under the kidney capsule were locally invasive, but tumors grown s.c. or intraperitoneally did not invade [16]. UCRU-PR-2 failed to grow when implanted in the liver or spleen; no experimental metastases were observed after intravenous injection. The sites of implantation did not result in the outgrowth of subpopulations as detected by light or electron microscopy, expression of tumor markers, or tissue expression of hormones. Electron microscopy revealed both glandular and neuroendocrine differentiation within the same cell, indicating trans-differentiation between the two different cell lineages. We were unable to establish a cell line in vitro in the absence of stromal cells but the line was maintained by in vivo passaging.

The karyotype of a G-banded metaphase of UCRU-PR-2 has been described [17]. It was hypodiploid and showed: 44XY, -1, +der(1)t(1;?)(p36;?), -2, +der(2), -6, -7, -10, -12, -13, +mar2, +mar3. The markers could not be clearly identified.

Subsequently our team's relocation to a new animal facility required that the line be re-established in mice from frozen tissue, but this was not successful and the line was lost.

UCRU-PR-4

This xenografted line has not been previously reported. The biopsy sample from which the xenografted UCRU-PR-4 was established was a moderately differentiated, cribriform adenocarcinoma of the prostate. Immunoperoxidase staining indicated that the tumor was positive for PSA (weakly) and PAcP and expressed cytokeratins (Fig. 1). The tumor nodule that formed from the initial xenografted tissue (UCRU-PR-4/0) was composed of a uniform population of small polyhedral cells aggregated in nests and islands and separated by fine connecting tissue septa. The tumor cells showed small uniform rounded nuclei with an effaced chromatin pattern and obvious nucleoli; the cytoplasm was scanty but eosinophilic. There was a vague suggestion of nuclear orientation into pseudorosettes but definite acini were not seen. The xenograft

TABLE I. Patient Details, Histological Findings, and DNA Flow Cytometry Profiles of Tumor Biopsies Which Grew in Nude Mice

Prostate Cancer Xenograft ID	Patient's age	Histology findings	Flow cytometry data: Ploidy	% G_0/G_1 cells (% S phase)
UCRU-PR-1	Not available	[a]Metastatic moderately differentiated prostatic adenocarcinoma	2.3N 4.6N	66 33
UCRU-PR-2	72	[b]Poorly differentiated adenocarcinoma of the prostate	2N 4N	82.3 (9.8) 7.9
UCRU-PR-4	71	[c]Diffusely infiltrating small acinar cell carcinoma plus more solid aggregates of poorly differentiated adenocarcinoma	2.4N[d]	Not available

[a]Xenograft was established from TURP. A metastasis in the patient's right knee was strongly positive for PCaP, but negative for PSA.
[b]With a past history of biopsy-proven benign prostatic hyperplasia, this patient presented with extensive prostatic cancer of the small acinar type. Bilateral orchidectomy caused an objective regression, and he remained well for 3 years. However, he relapsed with local recurrence and widespread metastases. Transurethral biopsy of the prostate revealed poorly differentiated prostatic cancer. It was this biopsy specimen that gave rise to the xenograft line, UCRU-PR-2. The patient was then treated with systemic estrogens, but without effect. He was not sufficiently fit for cytotoxic chemotherapy because of his advanced age, acute renal failure with metabolic acidosis, and poor general medical status. His condition deteriorated rapidly, and he subsequently died. Autopsy (and subsequent xenograft studies) revealed a small cell undifferentiated carcinoma of prostatic origin, with metastases in the bladder, ureter, liver, and lung. There was no evidence of an endobronchial primary small cell carcinoma.
[c]Patient presented 10 months earlier with urinary retention and a prostatic biopsy confirmed prostatic adenocarcinoma. He developed radiological evidence of widespread bony metastases and was given a short course of synthetic estrogen before proceeding to bilateral orchidectomy. He remained well for 10 months then re-presented with acute retention, and underwent transurethral resection. The xenograft was established from this TURP. The patient was subsequently lost to follow up.
[d]Xenograft showed generation of three different populations, 2.5N, 2N, 4.12N,

was considered to be histologically similar to that of the biopsy from which it was established and showed the same immunoprofile (Fig. 2). It was positive for EMA and cytokeratins 7 and 8 (CAM5.2) (see Fig. 1) and some cells were positive for PAcP, but negative for CEA and NSE and a range of neurosecretory polypeptides and generally negative or weakly positive for PSA. Some vimentin positive cells were seen in the glandular areas (not shown), suggestive of epithelial mesenchymal plasticity. These features support a very poorly differentiated adenocarcinoma, rather than undifferentiated small cell carcinoma in the xenograft. Subsequent passages of the xenograft (UCRU-PR-4/1 and 4/2) showed similar histology, with poorly differentiated adenocarcinoma, very occasional glandular acini, uniform round nuclei with a single nucleolus, eosinophilic cytoplasm and 1–2 mitoses/high power field.

Effects of Testosterone on UCRU-PR-4 Xenograft Growth

In order to examine the hormonal responsiveness of the xenografted lines, five nude mice were implanted with testosterone pellets at 60 days post tumor cell implantation, and tumor growth was compared with mice with no implants or following castration (5 per group). Plasma testosterone levels in mice were markedly increased when testosterone implants were used (30.6 ± 11.4 nmol/L) compared with untreated mice (1.3 ± 1.1 nmol/L). Levels in castrated mice were 1.5 ± 0.4 nmol/L. A higher tumor take rate was observed in mice implanted with the 1 cm testosterone implants (Fig. 2A) than in the absence of added testosterone (Fig. 2B). Castration of the mice after tumors reached 5×5 mm caused tumor regression in one mouse and stabilization of growth in another; one tumor started regrowing after castration (Fig. 2C) suggesting that populations of cells were heterogeneously androgen sensitive or castrate resistant. The tumors failed to grow when implanted in female nude mice. Cytogenetic analysis of UCRU-PR-4 was performed by direct analysis and following short term culture of xenografted tissue derived from the first passage of UCRU-PR-4. The karyotype shown in Figure 3 is 43–46, XY, dic(1;12)(p11;p11), der(3)t(3:?5)(q13; q13), -5, inv(7)(p15q35) x2, +add(7)(p13), add(8)(p22), add(11)(p14), add (13)(p11), add(20)(p12), -22, +r4[cp8]. The UCRU-PR-4 line failed to survive further passage.

DISCUSSION

Human tumor xenografts have been useful as preclinical models of cancers of bladder and prostate in our laboratories. In these two settings, there have been strong correlations between the human and

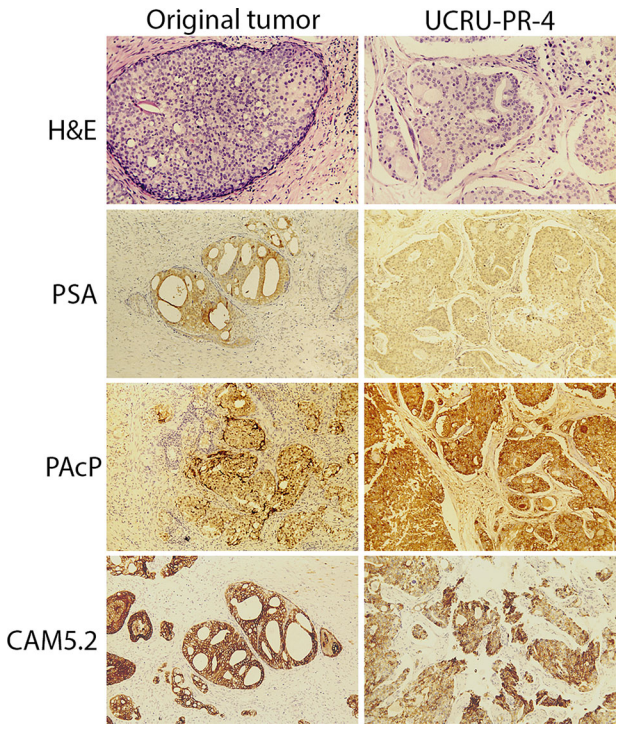

Fig. 1. Histological and immunohistochemical analysis of URCR-PR-4 donor material and xenograft (passage 1). Representative hematoxylin and eosin staining (H&E) and immunohistochemistry for prostate specific antigen (PSA), prostatic acid phosphatase (PAcP), and cytokeratins 7 and 8 (CAM 5.2) are shown.

xenograft phenotype and genotype, and the xenografts have reflected the human disease in responsiveness to therapy. In our bladder cancer studies, in particular, there were strong correlations between responsiveness to chemotherapy in xenografts and patients, and we were able to dissect the basis of patterns of cytotoxic resistance. Although UCRU-PR-1 was replaced by a murine tumor, karyotyping confirmed the human nature of the other two xenograft lines, and important potential correlations that may represent a genetic basis for human neuroendocrine cancers in prostate and lung.

We were not able to define whether the presence of aneuploid populations within the tumors was correlated with growth as xenografts in mice, as aneuploid or tetraploid cells were detected in all specimens. The samples that gave rise to transplantable xenografts (UCRU-PR-1, -2, and -4) were derived from aggressive advanced stage disease. In two cases (UCRU-PR-2 and UCRU-PR-4), the xenograft grew from tumors after the patient had received bilateral castration or systemic estrogen therapy, respectively. When implanted under the kidney capsule, UCRU-PR-2 was invasive [16], but did not form metastases.

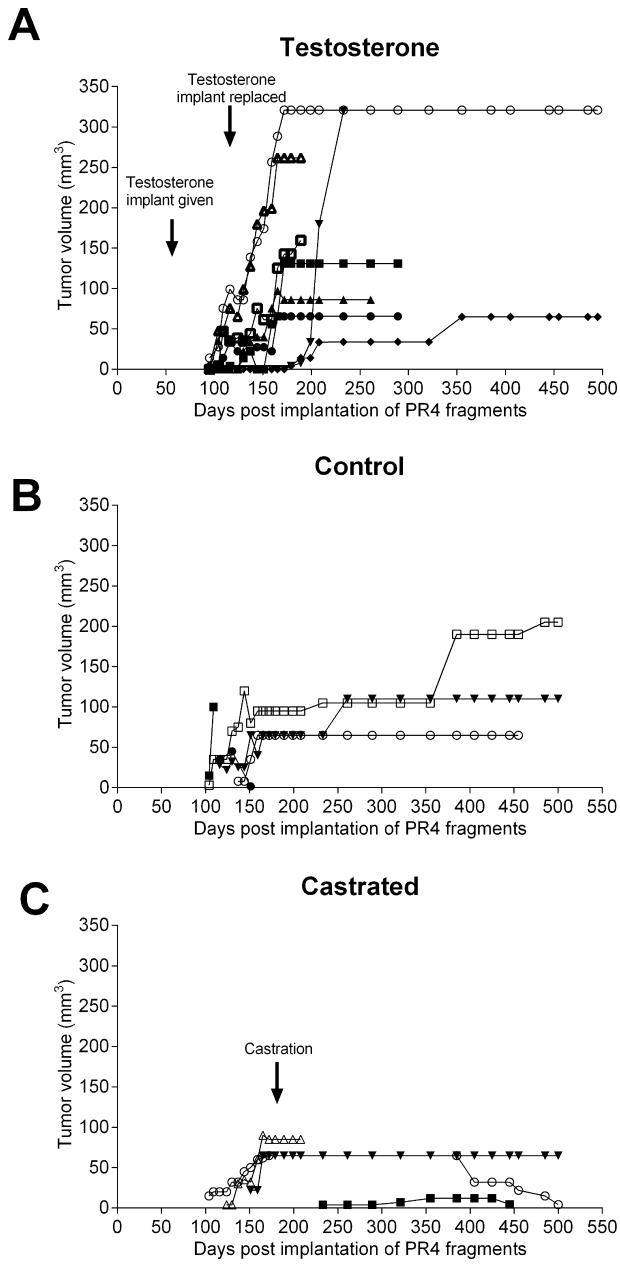

Fig. 2. Growth of URCR-PR-4 passage 1 xenografts in individual male mice **A**: supplemented with testosterone, **B**: controls (no intervention), and **C**: castrated. Timing of intervention is indicated.

The occurrence of neuroendocrine carcinoma in UCRU-PR-2, which was derived from a tumor that initially presented as an adenocarcinoma of the prostate, reflects what is now a well-described pattern – that is, an initial presentation with prostate adenocarcinoma and treatment by androgen deprivation therapy often antedates the first presentation of neuroendocrine cancer of prostate [28,29]. Of particular interest, the elaboration of hypothalamic-pituitary hormones presented a potential mechanism for autocrine control of growth of neuroendocrine

PCa. Given the presence of scattered neurosecretory elements in classical prostate adenocarcinomas, it is possible that the growth of these tumors may also be under partial autocrine regulation, a phenomenon that could be easily studied in the tissue-amplified xenograft environment.

We have shown that the UCRU-PR-2 xenografts responded to cisplatin, 4-epi-doxorubicin or adriamycin, each given individually at 5 mg/kg (data not shown), a pattern that reflects the cytotoxic responsiveness of human small cell anaplastic PCa [29]. Indeed, the high frequency of mixed neuroendocrine tumors with prostate adenocarcinomas has raised the question of whether combination therapy using hormonal therapy together with cisplatin or other drugs should be given initially to address both types of tumor [30].

The relationship between small cell carcinoma and adenocarcinoma of the prostate still remains obscure. There was previously controversy as to whether these tumors have a neuroendocrine origin alone, or whether they may represent a continuum extending from adenocarcinoma [31,32]. It was of interest that the karyotype described [17] from UCRU-PR-2 showed a loss of material from chromosome #10 described previously in PCa's of epithelial origin [33–35]. A similar marker to the 1p+ marker found in the UCRU-PR-2 line with a breakpoint at the site of the oncogene, *src* [36] was also described in a small cell carcinoma of the lung [37]. Morphological characteristics of xenografted large-cell neuroendocrine carcinomas (LCNEC) and small cell carcinomas derived from the same patient suggested that the LCNEC may be transitional between adenocarcinoma and SCCs [38]. It has also been suggested that snail transcription factor can regulate neuroendocrine differentiation in LNCaP PCa cells [39]. Recent data have implicated the repressor element-1 silencing transcription (REST) factor in the genesis of neuroendocrine differentiation [40] and have suggested that androgen deprivation or receptor blockade by the second-line agent, enzalutamide, may mimic REST inactivation leading to neuroendocrine differentiation [41].

The finding that testosterone pellets improved the take rate and increased growth in UCRU-PR-4 xenografts was of interest, given that the xenograft was derived from a CRPC tumor from a patient previously treated with estrogen therapy and bilateral castration. Unfortunately, we did not have studies of androgen receptor expression in this tumor. This response may reflect the differing systemic androgen status of mice and men. Intact male mice have circulating testosterone levels that approximate those of hypogonadal men [42]. This is an important consideration when using patient derived PCa xenografts. To model the testosterone levels of an aging human male one

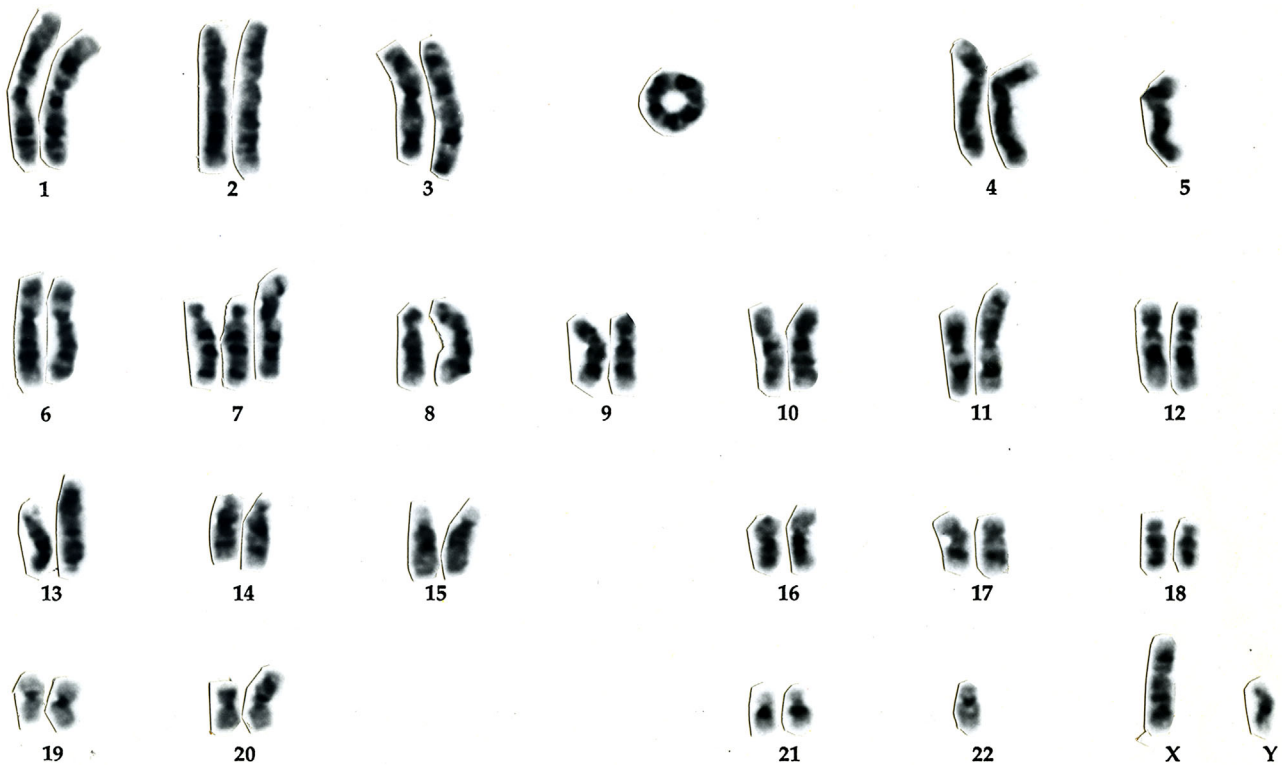

Fig. 3. URCR-PR-4 karyotype: 43–46, XY, dic(1;12)(p11;p11), der(3)t(3:?5)(q13;q13), -5, inv(7)(p15q35) x2, +add (7)(p13), add (8)(p22), add(11)(p14), add(13)(p11), add(20)(p12), -22, +r4[cp8].

would need to supplement mice with exogenous testosterone, and castrated mice most closely represent an abiraterone treated CRPC patient.

We have subsequently used both *scid* mice, which are more immunocompromised than *nude* mice, and various extracellular matrices (Matrigel^{TM}, collagen I) to provide additional support for the implanted PCa tissue. While this increased initial tumour take to approximately 90%, the generation of a serially transplantable adenocarcinoma xenograft has remained an uncommon phenomenon, although BM18, a transplantable xenograft derived from a bone metastasis was derived using this approach [43]. The LuCaP series of PCa xenografts developed by members of the Department of Urology at the University of Washington Medical Centre have also been established in SCID mice and have been generated from tumor material obtained from both primary and metastatic sites. A diverse spectrum of PCa phenotypes is represented amongst this panel [44], providing a useful platform for preclinical studies. More recent studies describe the use of even more immunocompromised mice, *nod scid gamma*, and implanting tissue under the kidney capsule, with the goal of providing a more vascularised graft site, to generate PCa xenografts [45,46].

Transplantable patient derived xenografts provide an excellent opportunity to capture some of the complexity, diversity and therapeutic responsiveness of clinical PCa. Features such as a spectrum of histological characteristics, responsiveness to androgens and relevant chemosensitivity enable preclinical modelling of the disease. Furthermore, new tools that may provide the ability to distinguish human and murine cells at the histological [43,47] or gene expression level [48] provide interesting opportunities to determine the contribution of the tumor cell and host stroma to the pathobiology underlying PCa.

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