

# Development and Characterization of Efficient Xenograft Models for Benign and Malignant Human Prostate Tissue

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**BACKGROUND.** Various research groups have attempted to grow fresh, histologically intact human prostate cancer tissues in immunodeficient mice. Unfortunately, grafting of such tissues to the sub-cutaneous compartment was found to be associated with low engraftment rates. Furthermore, xenografts could only be established using high-grade, advanced stage, but not low- or moderate-grade prostate cancer tissues.

**METHODS.** This paper describes methods for xenografting both benign and malignant human prostate tissue to severe combined immunodeficient (SCID) mice. We examine the efficiency and histopathologic consequences of grafting to the sub-cutaneous, sub-renal capsule, and prostatic orthotopic sites.

**RESULTS.** Sub-renal capsule grafting was most efficient in terms of take rate (>90%) for both benign and malignant tissue. Orthotopic grafts consistently exhibited the best histopathologic differentiation, although good differentiation with continued expression of androgen receptors (AR) and PSA was also seen in the sub-renal capsule site. Sub-cutaneous grafting resulted in low take rates and the lowest level of histodifferentiation in surviving grafts. Grafted benign tissues in all sites appropriately expressed AR, PSA, cytokeratins 8, 18, and 14 as well as p63; carcinoma tissues did not express the basal cell markers. Grafting of tissues to castrated hosts did not affect

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Abbreviations: SCID, severe combined immunodeficient; AR, androgen receptors; PSA, prostate specific antigen; BPH, benign prostatic hyperplasia; PIN, prostatic intraepithelial neoplasia; CaP, carcinoma of the prostate; UCSF, University of California, San Francisco; VUMC, Vanderbilt University Medical Center; H&E, hematoxylin and eosin; BCCA, British Columbia Cancer Agency; DME, Dulbecco's modified eagles (medium); PBS, phosphate buffered saline; DAB, 3,3'-diaminobenzidine tetrahydrochloride; IDCa, intraductal carcinoma; T, testosterone; SV, seminal vesicle; AP, anterior prostate.

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the graft take rates (but was not practical in the case of the orthotopic site). Grafting followed by host castration resulted in epithelial regression with loss PSA and reduced AR expression at all three sites.

**CONCLUSIONS.** These data suggest that sub-renal capsule and orthotopic grafting of human prostate tissue can be used for many basic scientific and translational studies. *Prostate* 64: 149–159, 2005. © 2005 Wiley-Liss, Inc.

**KEY WORDS:** prostate cancer; benign prostate; sub-renal capsule; orthotopic site; sub-cutaneous site; xenograft

## INTRODUCTION

Translational research into the progression and treatment of prostate cancer has been hampered by the relative paucity of *in vivo* models of the disease. The processes of carcinogenesis and tumor progression are complex and are the result of changes within the epithelial cells; interactions occurring both between the stromal and epithelial tissues of the tumor; and, between the tumor as a whole and the many local and systemic influences found in the host. Systemic influences include factors such as immune response to the tumor and hormonal status of the host. Local influences include factors such as the tissue type in which the tumor is developing, adjacent tissues and, in metastasis, interactions between the metastasizing cell, and the tissue type into which it is invading.

Any model system will, by its very nature, be limited in its ability to address all of these complex relationships. *In vivo* xenografting of tissue fragments allows many issues to be examined. This methodology has been used by a number of groups but has historically proven unreliable when applied to human prostate cancer tissues [1–7]. While a number of transplantable human prostate tumor lines have been successfully established, these have represented a small fraction of the total number of tissue fragments which have been grafted [2,3]. The purpose of the present study was not to establish transplantable tumor lines (although we certainly recognize that these approaches can be modified in this direction) but rather to determine whether a highly efficient method could be devised for maintaining individual low- to mid-grade human prostate tumors *in vivo* while maintaining a differentiation profile consistent with the originally grafted tissue. Such a model would allow many new investigations to proceed, these include: studies on the effects of potential therapeutic agents on human prostate tumor tissues *in vivo*; pharmacogenomic and proteomic studies; fundamental studies on basic aspects of tumor biology; and investigation of genes regulated in human prostate tissue by androgenic stimulation *in vivo*.

Xenografting studies have historically used three major graft sites; sub-cutaneous, sub-renal capsule, and orthotopic [4,8–10]. Each of these has its own

advantages and disadvantages. The sub-cutaneous site is easily accessible and has a high capacity, however the site is poorly vascularized and reported take rates for primary tumors have generally been low [7]. The sub-renal capsule site is highly vascularized with an associated very high take rate for most grafted tissues including benign human prostate tissue [10]. However, the surgery is more technically demanding than for the sub-cutaneous site and the xenograft carrying capacity is lower than for sub-cutaneous grafting. Orthotopic grafting has the perceived advantage that the site is representative of the environment in which the tumor originated [11–14]. In the case of human prostate tumors grafted into rodents, a strong case could be made either for or against this contention. However, at a practical level, the main disadvantages of orthotopic grafting into the prostate of rodents are surgical access to the site and limited xenograft carrying capacity. Androgen ablation of rodents results in such extreme prostatic shrinkage that subsequent orthotopic xenografting becomes impractical, limiting the use of this site in some applications. Despite these considerations, the orthotopic site of the intact mouse was examined in this study.

Xenografting requires the use of immunocompromised hosts. The absence of an immune system is one of the major inherent limitations of this class of model. Given concerns that have been expressed over the ability of athymic animals to generate a limited immunologic response to some human tumors, it was decided to utilize severe combined immunodeficient (SCID) mice as hosts for the present study [15].

Androgenic stimulation is a key component of prostatic biology. Without androgens the prostate does not develop. Androgen ablation during adulthood leads to the induction of apoptosis and a rapid involution of the gland [16]. We have previously demonstrated that benign human prostatic tissue xenografted to the renal capsule of athymic mouse hosts undergoes such an apoptotic response following castration of the host [10]. Given that concerns have been expressed relating to the ability of endogenous levels of androgens seen in mice to support the growth of xenografted human prostate cancer, it was decided to perform these studies (except where specifically indicated) in

castrated mice supplemented with testosterone implants [17]. This provides a uniform high androgen titer in the mice.

The present communication describes the efficiency and histologic consequences of grafting benign and malignant human prostate cancer tissues into the sub-cutaneous, sub-renal capsular, and orthotopic sites. The effects of androgen regulation on tissues at the three sites are also described. We demonstrate here that it is possible to graft low-grade human prostate tumor samples at extremely high efficiency to SCID mouse hosts opening the way for new pharmacologic, pharmacogenomic, and biological studies.

## MATERIALS AND METHODS

### Prostate Tissues

In order to determine the general applicability of this technique, we utilized tissues from three sources. In particular this was to test the efficacy of this method with both radical prostatectomy and needle biopsy samples. Twelve cases of benign prostatic hyperplasia (BPH) and 12 specimens of prostate tissue containing low-grade, Gleason score 6 (3+3), prostate cancer lesions were obtained from radical prostatectomies (untreated primary carcinoma) supplied by Department of Pathology, UCSF. Nine prostate cancer and three benign samples were obtained from the VUMC, Department of Pathology via the Vanderbilt-Ingram Cancer Center Tissue Acquisition Core. Five additional low-grade specimens (Gleason score 6) from needle biopsies were supplied by the Department of Urology, Gulf View Medical Center, La Romaine, Trinidad. Histology of the tissue prior to grafting was established by analysis of H&E stained frozen sections adjacent to the samples to be grafted.

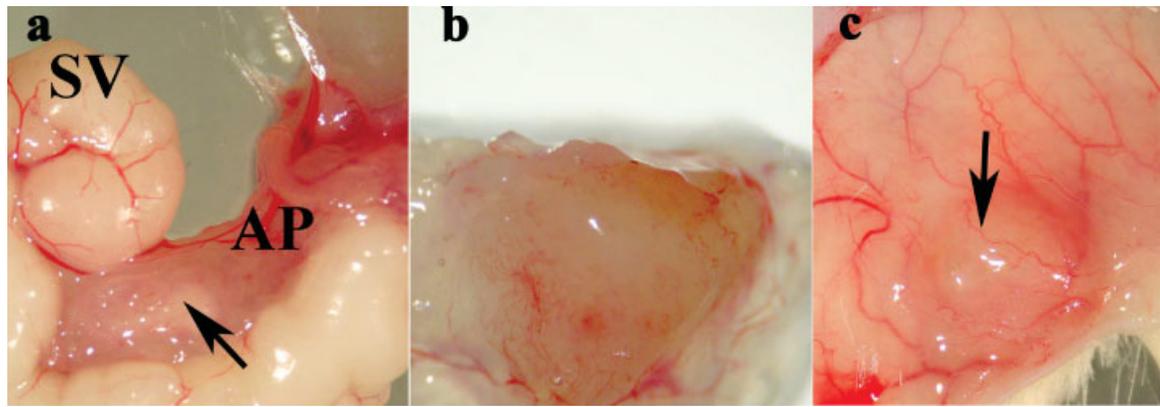
### Xenografting

Tissues were collected in DME H16 50%/Ham's F12 50% media mix (Life Technologies), containing penicillin (100 units/ml; Life Technologies), and streptomycin (100 mg/ml; Life Technologies) within 60 min of removal from the patients. Depending upon the source of the tissues (UCSF, Vanderbilt or Trinidad) and the site of grafting (UCSF, Vanderbilt or BCCA) tissues were used directly on site or shipped overnight on wet ice. Tissues were cut into small (3 × 3 × 3 mm) pieces with a scalpel. Representative fragments were fixed in formalin and processed to paraffin to confirm histology. The needle biopsies were cut into 3 mm blocks (3 mm long × 1 mm in diameter) and processed in the same manner as the prostatectomy samples. Note that since individual samples were cut into multiple fragments before grafting the number of grafts recorded differs from the number of patients.

One group of specimens was grafted under the renal capsule, another group was grafted sub-cutaneously, and a third orthotopically into the anterior prostate of the same male SCID mice (Charles River, Wilmington, MA). An illustrated tutorial for the performance of sub-renal capsule grafting, with illustrations of the appearance of representative grafts can be found at: <http://mammary.nih.gov/tools/mousework/Cunha001/index.html>. Sub-cutaneous grafting was performed via a midline dorsal incision. Tissue samples were inserted beneath the dorsal skin and the wound sealed with clips. For orthotopic grafting a 1-cm midline ventral incision was made starting above the bladder. The seminal vesicles and anterior prostate were exteriorized. The two main ducts of the anterior prostate running inside the coil of the seminal vesicle were identified. A 2–3 mm incision was made between the ducts. Using a fire-rounded glass pipette tip, a pocket was formed to receive the graft. The graft was inserted, the organs replaced, and the body wall and skin closed. Figure 1 illustrates the gross appearance of orthotopically and sub-cutaneously grafted tumor samples. The hosts were castrated and routinely supplemented with a 1-cm silastic capsule (Catalog no. 602-305, Dow-Corning Co., Midlands, MI) filled with 6 mg testosterone. Grafts were collected after growth for 30 or 90 days. In cases where the effects of androgen ablation were studied, the silastic implants were removed.

### Histopathology and Immunohistochemistry

The original samples and the harvested grafts were fixed in 10% neutral buffered formalin, and processed to paraffin. Sections were cut on a microtome and mounted on glass slides. Sections were de-waxed in Histoclear (National Diagnostic, Atlanta, GA) and hydrated in graded alcoholic solutions and distilled water. For histopathology, routine H&E staining was carried out. For immunohistochemistry, endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in methanol for 30 min followed by washing in phosphate buffered saline (PBS) pH 7.4. Five percent normal goat or donkey serum in PBS (as appropriate) was applied to the sections for 30 min to bind non-specific sites. The sections were then incubated with the primary antibodies overnight at 4°C or with non-immune mouse IgG. A rabbit polyclonal anti-AR antibody (PA1-111A) was purchased from Affinity BioReagents (Golden, CO). Anti-cytokeratin antibodies, all mouse monoclonals (LE41, LE61, LP34, and LL001 against keratins, 8, 18, 5, and 14, respectively, [18–20]) were generously provided by Dr. E.B. Lane, University of Dundee. (Note that LP34 recognizes a broad spectrum of keratins, however, it most strongly reacts with keratin 5 and is basal cell-specific in normal



**Fig. 1.** Orthotopic and sub-cutaneous xenografts of human prostate tissue in a SCID mouse. **a:** Wholemount showing the anterior prostate or orthotopic graft in place. The anterior prostate is seen in the “crook” of the seminal vesicle (SV). The graft (arrow) can be clearly seen nestled between the two main ducts of the anterior prostate (AP). **b:** Gentle microdissection reveals the graft which has clearly become extensively vascularized. **c:** Wholemount view of a sub-cutaneous graft beneath the skin of a SCID mouse.

human and rodent prostate.) An antibody raised against smooth muscle  $\alpha$ -actin (clone 1A4) was purchased from Sigma. A polyclonal rabbit antibody raised against PSA was purchased from Dakopatts (Carpinteria, CA). Anti-p63 antibody (clone N-16) was purchased from Santa Cruz Biotech (Santa Cruz, CA). Anti-Ki67 antibody (clone MIB-1) was purchased from Immunotech (Westbrook, ME). Purified rabbit and mouse IgGs were obtained from Zymed Corp. (So. San Francisco, CA). Biotinylated anti-rabbit and anti-mouse IgGs were obtained from Amersham International (Arlington Heights, IL). Biotinylated anti-goat antibody was purchased from Sigma. Peroxidase linked avidin/biotin complex reagents were obtained from Vector Laboratories (Burlingame, CA). Following incubation with the primary antibodies, sections were washed with PBS and incubated with the appropriate biotinylated secondary anti-mouse immunoglobulin diluted with PBS at 1:200 for 30 min at room temperature. After incubation with the secondary antibody, sections were washed in PBS (three 10 min washes), and then incubated with avidin–biotin complex (Vector laboratories, Foster City, CA) for 30 min at room temperature. Following a further 30 min of washing in PBS, immunoreactivity was visualized using 3,3'-diaminobenzidine tetrahydrochloride (DAB) in PBS and 0.03%  $H_2O_2$ . Sections were counterstained with hematoxylin, and dehydrated in graded alcohols. Control sections were processed in parallel with mouse or rabbit non-immune IgG (Dako) at the same concentration as the primary antibodies.

#### Ki 67 Labeling Indices

Before and after grafting tumor and benign samples were stained for Ki67 immunoreactivity and examined to determine the proliferation index. The Ki67 label-

ing index, as a measure for proliferation, was assessed by counting labeled and unlabeled epithelial cells in each sample at 20 $\times$  magnification microscopic field. The percentage of Ki67 positive cells was calculated as the proliferation index.

#### Histology of Grafted Tissues

The samples were assessed by light microscopy to determine percentage of glands and stroma, extent and severity of atrophy, presence of inflammation in glands or stroma, presence or absence of basal cell hyperplasia, presence and type of metaplasia (squamous or transitional), and the percentage and grade of the tumor present in the graft. Atrophy was considered when the epithelial cells were flattened, and the glandular lumen was dilated.

The histologic features were assessed as percentage and semi-quantitatively as follows: 0 = absent; 1+ = <25% of the sample (mild); 2+ = >25% to 50% (moderate); 3+ = >50% (severe).

## RESULTS

#### Efficiency of Viable Graft Recovery

In line with our previous experience for benign tissues [10], the sub-renal capsule site was found to be extremely efficient with nearly 95% of grafts recovered (Table I). This reflects to some extent the experience level of the various individuals performing the surgery but does make the point that it is possible to recover the vast majority of grafts into this site. Efficiency at the anterior prostatic orthotopic site was also high (in excess of 70% of grafts were recovered). In contrast to the sub-renal capsular and orthotopic sites the take rate of sub-cutaneously grafted tissues was around 50%, a rate higher than previously reported for human

**TABLE I. Overall Recovery Rates of Grafted Human Prostate Tissues at the Sub-Renal Capsule, Prostatic Orthotopic, and Sub-Cutaneous Sites**

Grafting sites	Total grafts implanted	Total grafts harvested	Take rate (%)
Sub-renal capsule	122	114	93.4
Sub-cutaneous	86	50	58.1
Orthotopic	57	41	71.9

Recovery rates of benign versus malignant tissues were not significantly different at any given site.

prostate cancer tissue but still lower than that seen in the other sites. The recovery rates of grafts of benign and malignant tissues were essentially identical.

In a small study grafting to the renal capsule and sub-cutaneous sites of castrate animals the take rate was in line with that seen in testosterone implanted hosts. Orthotopic grafting to castrate animals was found to be impractical due to the small size of the castrate mouse prostate.

#### Histopathology of the Recovered Benign Prostatic Tissues

To compare histologic responses to the graft site, benign prostate tissues were used as a first comparison. These tissues have less intrinsic internal heterogeneity than prostate cancer tissues enabling a more rigorous comparison. Tissues were harvested and compared at 1 and 3 months post-grafting.

In benign tissues, the extent and severity of atrophic changes was very mild at the orthotopic site at 1 month; mild to moderate at the sub-capsular site and moderate to severe at the sub-cutaneous site. There was increase in atrophic changes in the orthotopic site at 3 months to the mild to moderate range. The sub-capsular and sub-cutaneous sites did not show significant increase in atrophic changes at 3 months post-grafting.

The ratio of glands to stroma in tissues grafted to the different sites was not significantly different at one month post-grafting. At 3 months post-grafting in castrated T-supplemented hosts significantly more glands were seen at the orthotopic site, compared to the renal capsule and sub-cutaneous sites ( $P = 0.06$  and  $0.02$ , respectively, by *t*-test comparison).

A composite photomount illustrating typical benign grafts at the three sites is presented in Figure 2. Mild to moderate basal cell hyperplasia was noted in the orthotopic and sub-cutaneous sites at 1 month post-grafting with mild decrease of hyperplastic change at 3 months in castrated and T-supplemented hosts. The

renal sub-capsular site showed minimal basal cell hyperplasia at 1 and 3 months post-grafting.

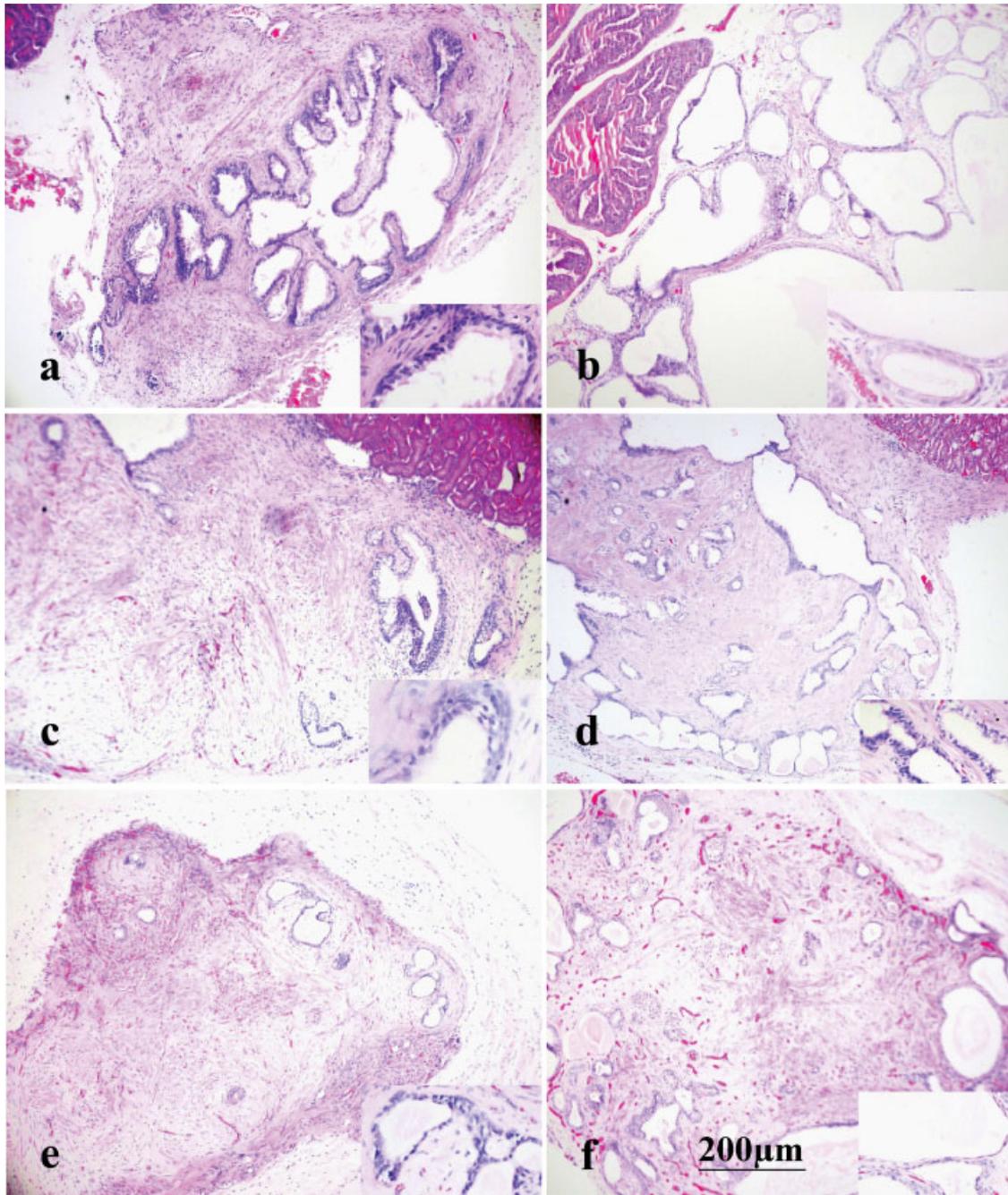
Stromal inflammation was very mild in the orthotopic site and was mild to moderate in the sub-capsular and sub-cutaneous grafts at 1 and 3 months post-grafting. Gland inflammation was rare in all sites at any time post-grafting.

One in 10 (1/10) orthotopic samples at 1 month post-grafting showed transitional metaplasia. The sub-cutaneous grafts at 1 (7/10) and 3 months (3/4) showed squamous epithelial metaplasia. The orthotopic grafts at 3 months and the renal grafts at 1 and 3 months did not show epithelial metaplasia.

#### Histopathology of the Recovered Prostate Tumors

Clearly it is possible that benign and malignant tissues will behave differently in graft sites and for this reason we also compared grafts of tumor tissue to sections of adjacent source tissue obtained prior to grafting. Histopathology of human prostate cancer xenografts was assessed prior to grafting and after growth in SCID mouse hosts. Seventeen human prostate tissue samples containing low-grade human prostate cancer (Gleason grade 3) were examined following growth under the renal capsule of castrated SCID mice supplemented with testosterone. These samples were obtained from either needle biopsies or from radical prostatectomies (each graft tissue is about  $1 \times 3 \times 3$  mm). Figure 3 shows a human prostate cancer specimen of low Gleason grade before and after sub-renal capsule grafting. The histopathology of the human prostate cancer graft did not change during the growth period in the mouse host (Fig. 3a,b). The proliferation rate, as judged by Ki67 immunoreactivity, was similar in the carcinoma samples before  $2.49\% \pm 0.40\%$  ( $n = 9$ ) and after grafting  $2.28\% \pm 0.48\%$  ( $n = 8$ ) (Fig. 3c,d). PSA, a differentiation marker, was strongly expressed in the human prostate cancer graft (Fig. 3f) grown in T-supplemented hosts as well as in the specimen before grafting (Fig. 3e). Thus, human prostate cancer transplants maintained a similar histopathology, differentiation status, and proliferation rate to that of the corresponding donor tissue, even after being passaged three times through new hosts over a 6-month growth period.

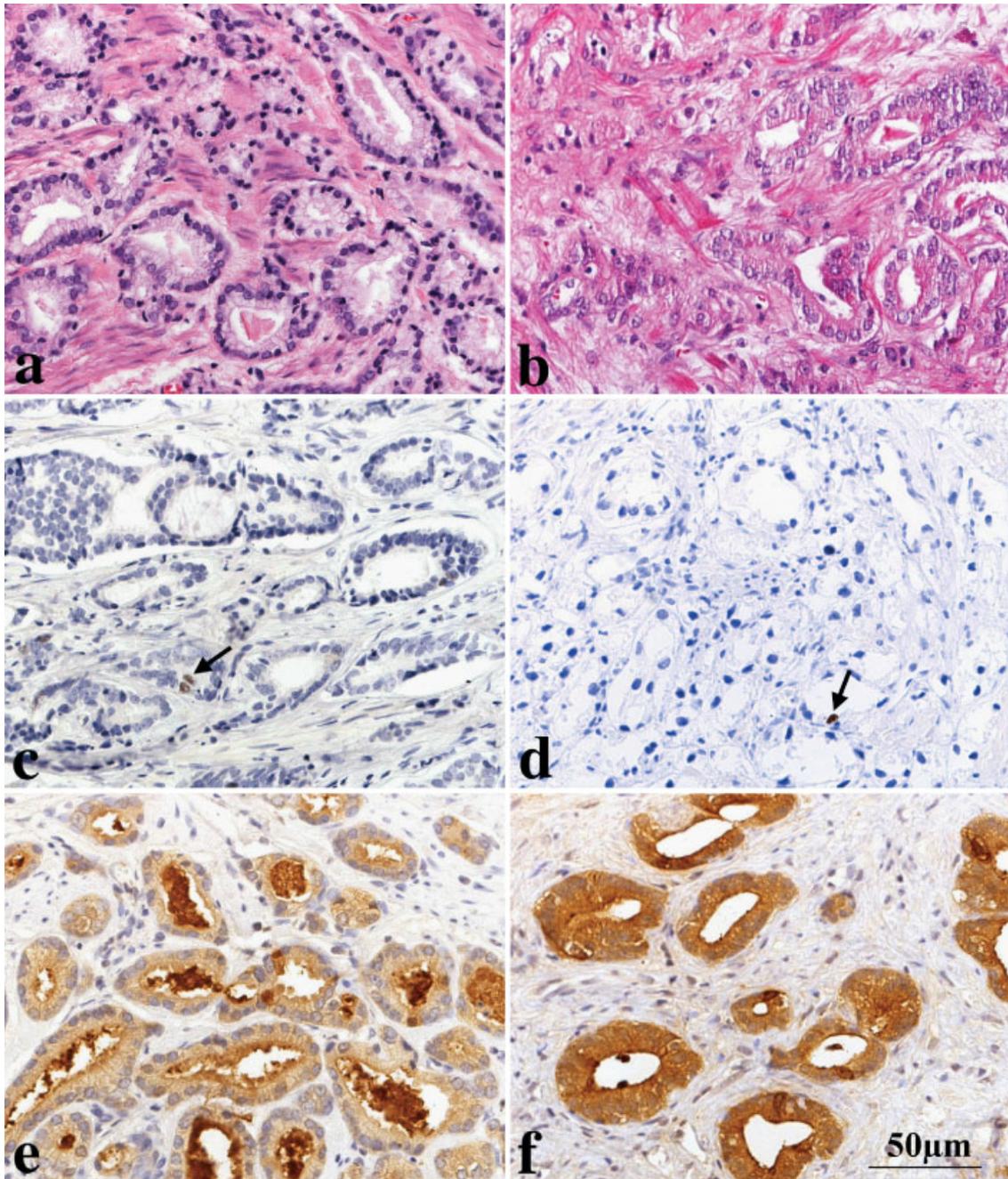
While it is relatively straightforward to sample either benign or tumor tissue, the grafting of small pre-malignant lesions requires a degree of luck and is presented here to demonstrate that this is possible. Figure 4 shows an example of a PIN lesion before (Fig. 4a) and after 90 days of growth in a T-supplemented castrated SCID host (Fig. 4b). This particular PIN lesion before grafting (Fig. 4c) had a partially disrupted basal cell layer, as judged by loss of



**Fig. 2.** Histology of benign human prostate at the orthotopic, sub-renal capsule, and sub-cutaneous graft sites of castrated T-supplemented SCID mouse hosts. Morphologic features at the orthotopic (a and b), sub-capsular (c and d), and sub-cutaneous (e and f) sites at 1 (a, c, e) and 3 months post-grafting (b, d, f). The glands at the orthotopic site appear well preserved with no atrophic changes. The glands at the sub-capsular site demonstrate slightly more developed atrophic changes with flattening of the epithelium and scant stromal inflammation. The glands in the sub-cutaneous site have more marked atrophy with dilated glands, flattened epithelium, and significant stromal fibrosis. The changes are slightly more prominent 3 months post-grafting. (H&E stain 20 $\times$ ). In the inset pictures in the inferior corners, a higher magnification of the morphologic features of the epithelial changes are seen.

p63 immunostaining that was continuous with normal prostatic epithelium having a continuous p63 positive basal cell layer. The disrupted basal cell layer was also observed in this particular PIN lesion after 3 months of

growth in a T-supplemented host (Fig. 4d). PSA was detected in this PIN lesion by immunohistochemistry before (Fig. 4e) and after *in vivo* growth under the renal capsule (Fig. 4f).



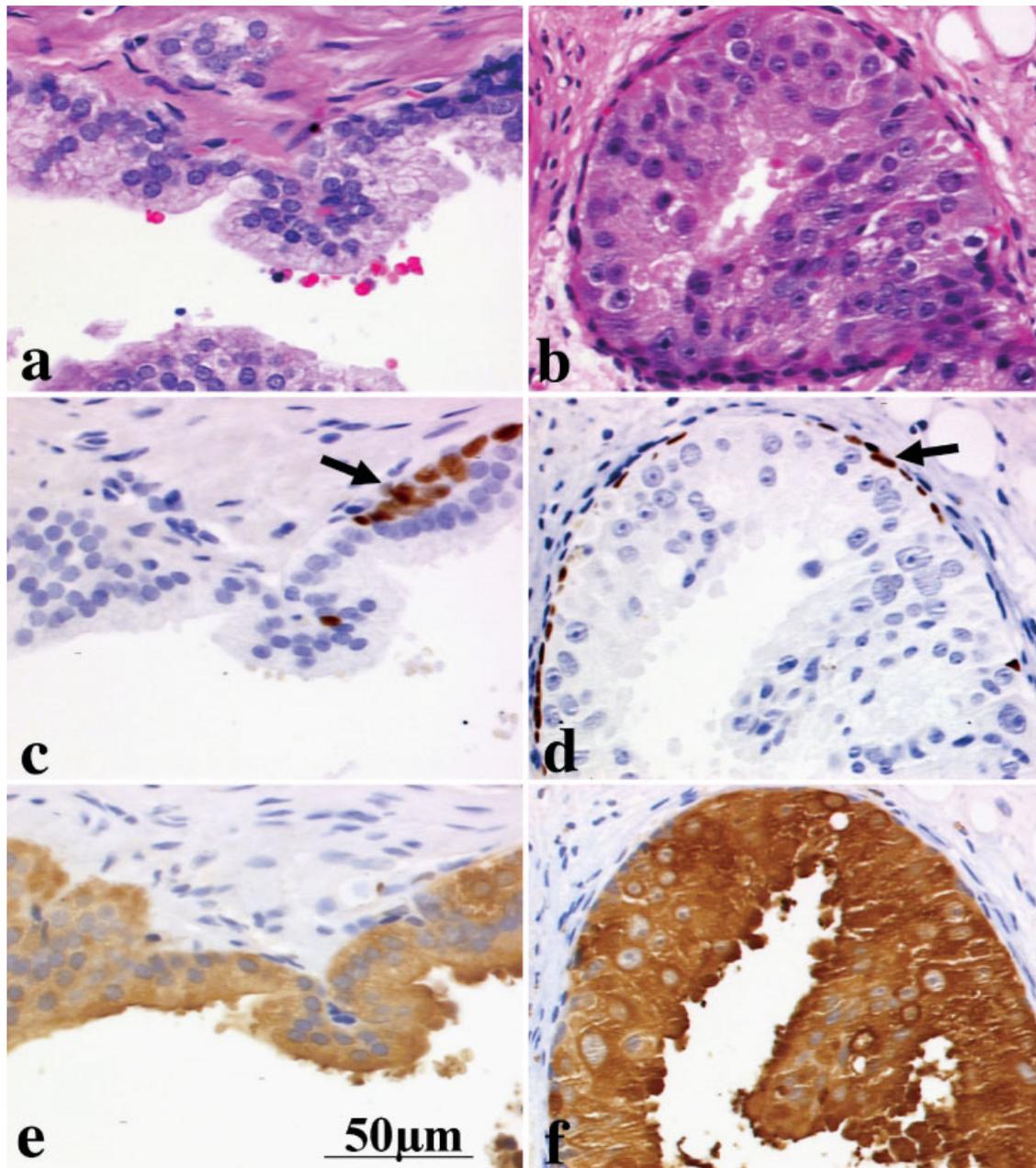
**Fig. 3.** Gleason grade 3 human prostate cancer specimen before (a, c, and e) and after 3 months of growth in a T-supplemented castrate male SCID host (b, d, and f). Gleason grade histopathology (a, b), proliferation as indicated by Ki67 (c, d), and differentiation as indicated by PSA (e, f) of grafted tumors resemble the original lesion (a, c, and e).

#### Pathology of Tissues to Intact Versus Castrated Hosts

Figure 5 illustrates the effects of androgen deprivation on benign and malignant human prostate tissue grafted to SCID mouse hosts. Benign human prostate tissue grafted beneath the renal capsule of castrated mice regressed demonstrating flattening and meta-

plastic changes of the sort associated with androgen ablation in human patients (Fig. 5b). Secretory differentiation was maintained in matched samples grafted to testosterone supplemented animals (Fig. 5c).

The response to androgen ablation seen with benign tissue grafts was paralleled with cancer tissue. Panel 5d shows part of the original cancer tissue core from which the grafted samples in panels 5e and 5f were derived.



**Fig. 4.** Prostatic tissue with prostatic intraepithelial neoplasia (PIN) lesions before (**a**, **c**, and **e**) and after growth for 3 months under the renal capsule of T-supplemented castrate male SCID hosts (**b**, **d**, and **f**); (**a** and **b**) hematoxylin and eosin staining; (**c** and **d**) p63 staining; and (**e** and **f**) PSA staining. Note the discontinuity of the p63-positive basal cell layer (arrows in **c** and **d**). PSA staining is similar in the PIN lesion before and after growth in a T-supplemented male SCID host.

Histologically, fused glands indicative of Gleason pattern 4 (bottom left and center) are shown admixed with larger circumscribed cribriform formations compatible with intraductal carcinoma (IDCa), a lesion recently recognized as constituting the spread of invasive carcinoma within ducts and conferring adverse prognosis [21–24]. Panel 5e shows tissue which has been grown for 1 month to a castrated SCID mouse,

showing regression of the tumor, with a shrunken cribriform focus demonstrating pyknotic nuclei and absence of conspicuous nucleoli. In contrast, the tissue grown for 1 month in a testosterone supplemented castrated SCID mouse has a histopathologic appearance similar to the core from which it was derived (Fig. 5f). Other more usual acinar forming prostate carcinomas (i.e., Gleason pattern 3) have shown glands

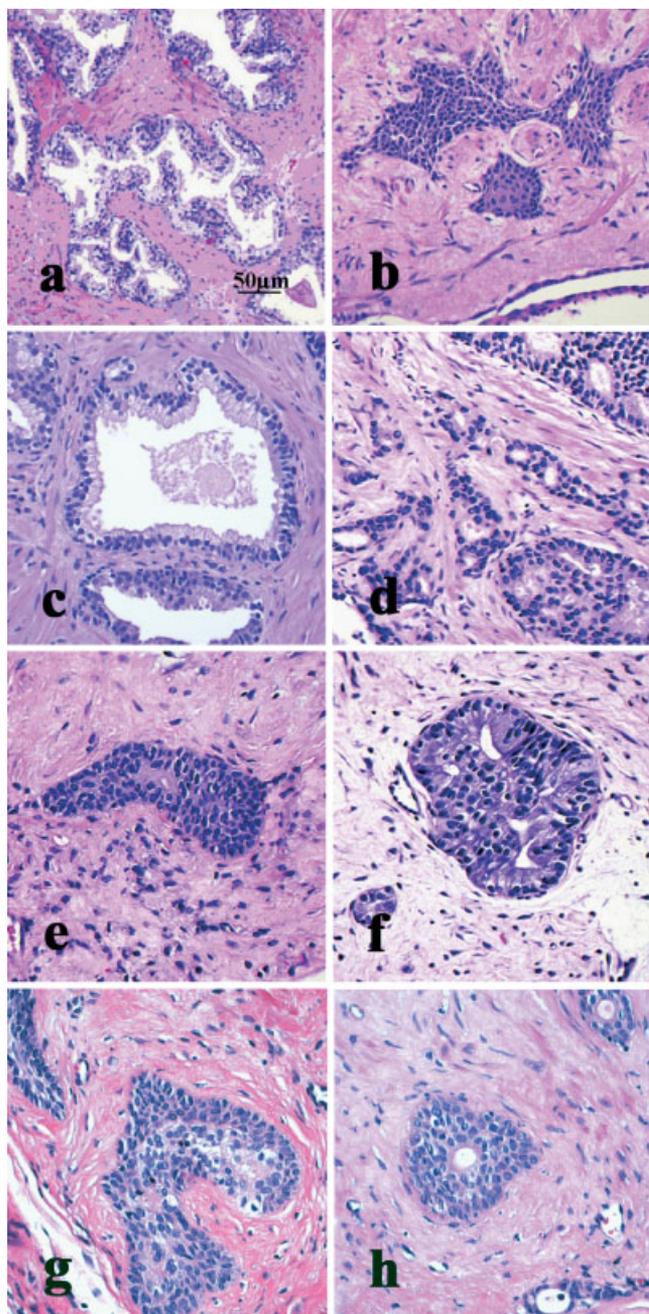
with clear cytoplasm and pyknotic nuclei when implanted in androgen ablated hosts. Similar phenotypic changes in response to androgen ablation were also seen in tissues grafted to the orthotopic (Fig. 5g) and sub-cutaneous (Fig. 5h) sites. This is similar to well-described alterations of Gleason pattern 3 (Gleason score  $3 + 3 = 6$ ) tumors in response to standard neoadjuvant hormone deprivation treatment in patients [25,26], without such changes evident in testosterone supplemented hosts (not shown). These data demonstrate that human prostate cancer tissue can be effi-

ciently grown in SCID mouse hosts where it can respond to the hormonal environment provided by the host.

## DISCUSSION

In order to perform pharmacogenomic and drug response studies it is extremely useful to have *in vivo* xenograft models in which the response of specific patient samples to a given therapeutic regimen can be monitored *in vivo* and then compared to molecular characteristics, such as genomic or proteomic profiles, of the source tissue taken directly from the patient. In this regard *in vivo* models of human tissues which retain appropriate stromal–epithelial interactions clearly present a more accurate picture of organ and disease biology than isolated cultured cell populations. Many of the most important systemic influences on prostate tissue are the result of paracrine interactions acting under the overall control of the endocrine system [27]. This study characterizes a highly efficient method by which benign and malignant human prostate tissues can be maintained allowing new approaches for studying human prostate biology especially that of low-grade prostate cancer in an *in vivo* environment.

Over the past two to three decades, large numbers of clinical prostate cancer samples have been grafted sub-cutaneously into athymic or SCID mice [7]. Most implants of low-grade human prostate cancer specimens have failed to survive [1–5]. Even for advanced prostatic lesions, the take rate in the sub-cutaneous site of immunodeficient mice has been very low [2]. A limited number of mostly advanced human prostate



**Fig. 5.** Effects of androgen ablation on benign and cancer xenografts to the renal capsule site. **a:** Benign tissue core from which **(b)** and **(c)** were derived. **b:** Benign human prostate tissue grafted beneath the renal capsule of castrated and castrated, testosterone supplemented **(c)** SCID mice. Note the regressed state of the graft to the castrate host with flattening and metaplastic changes associated with androgen ablation in human patients. In contrast secretory differentiation was maintained in the testosterone supplemented animal **(c)**. **d:** It shows part of the original tissue core from which the grafted samples shown in **(e)** and **(f)** were derived. Fused glands indicative of Gleason pattern 4 (bottom left and center) are shown admixed with larger circumscribed cribriform formations compatible with intraductal carcinoma (IDCa). **e:** It shows tissue which has been grown for 1 month in a castrated SCID mouse, demonstrating regression of the tumor, with a shrunken cribriform focus demonstrating pyknotic nuclei and absence of conspicuous nucleoli. In contrast **(f)** was grown for 1 month in a testosterone supplemented castrated SCID mouse. The tumor has a histopathologic appearance similar to the core from which it was derived **(d)**. Grafts to the orthotopic **(g)** and sub-cutaneous **(h)** sites also responded to androgen withdrawal with regression consistent with that seen at the renal capsule **(e)**.

cancers (stages C and D) have been successfully propagated to generate xenograft lines [3,6,7,28–30]. While many aspects of prostate cancer biology can be successfully modeled in such transplantable tumor models, they have limited applicability in determining the variability of responses to treatment regimens, which can be expected across wider populations or in the development of individual patient-based pharmacogenomics. Therefore the aim of this study was not specifically to generate transplantable lines, although the methods employed here can be modified to be used as a basis for generating such lines.

The use of various grafting sites by different groups has been based largely upon the experience of those groups. In the present study, we provide side by side comparison of the relative efficiency of three graft sites (sub-cutaneous, renal capsule, and orthotopic). This study demonstrates, using matched samples in the same host, the high efficiency of the sub-renal capsular site in the hands of experienced operators as compared to the technically much simpler sub-cutaneous graft site. We have previously speculated that the high efficiency of the sub-renal capsular site is results from its high degree of vascularity as compared to the relatively low level of blood vessels found sub-cutaneously. The use of the orthotopic prostatic site for xenografting of tissues (as distinct from injections of cell suspensions) has not been widespread, due largely to the technical difficulties in reaching and implanting tissue in this location [8]. The present study demonstrates that, at least in skilled hands, it is possible to graft human prostate tissue to the mouse anterior prostate with acceptably high efficiency. The major limitation of this site is the small volume of tissue which can be placed here.

Since prostate cancer tissue is, by its very nature, highly heterogeneous, we initially assessed the effects of graft site on tissue histology using benign prostate tissue, which has a much more consistent phenotype. The histology of the harvested tissues was found to be best in the orthotopically grafted samples and was also good in the renal capsule implants. Sub-cutaneously grafted tissues had the poorest profile of histopathologic differentiation. Analysis of tumor samples also showed strong similarities between the source tumor and grafts to either the orthotopic or renal capsule site. Given the inherent variability of human prostate tumors it is difficult to determine which of these two sites is superior in reflecting the tumor biology seen in the patient.

The use of orthotopic graft sites has been suggested to represent the best approach to modeling as the site is thought to provide the key characteristics of the organ of origin which may well be important in establishment and progression of disease. In particular, the use of

orthotopic sites versus other sites may well be important in the metastatic spread patterns of any advanced malignant graft. No metastasis has been seen in the model systems representing low-grade prostate cancer presented here. This likely reflects the slow growth and spread of low-grade human prostate cancer. It is also important to consider in a hypothetical metastatic model whether the difference between a rodent and human host are important. For example it is possible that the pre-dilection of human prostate cancer to metastasize to bone may reflect some unique property of the human bone environment which is not recapitulated in a mouse host as suggested previously [31,32]. Thus studies of metastasis in such xenograft models might require the incorporation of human bone targets.

We have previously described responses of human benign prostate tissue to androgen ablation in both sub-cutaneous and sub-renal capsule grafting models [10,33]. The present study confirms that similar changes also occur at the orthotopic site. This observation confirms the ability of human prostate cancer xenografts to respond appropriately to hormonal stimuli in these *in vivo* models, and raises the possibility that these models will respond to pharmacologic agents in a manner which parallels the response profile seen in human patients.

The ability to grow samples of human prostate cancer with high efficiency as described here opens the doors to many new possibilities. These include investigations of basic biological aspects of human prostate cancer in an *in vivo* environment, and especially the development of translational and pharmacogenomic studies across expanded patient population bases.

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