WISH-PC2: A Unique Xenograft Model of Human Prostatic Small Cell Carcinoma


Department of Immunology [J. H. P., T. W., D. G. S., Z. E.] and The Experimental Animal Center [A. H.], The Weizmann Institute of Science, Rehovot 76100, Israel; Department of Urology [J. H. P., J. R.], Sheba Medical Center, Tel-Hashomer 52621 Israel; and Departments of Pathology [J. W. S.] and Urology [A. B.], UCLA School of Medicine, Los Angeles, California 90095

Abstract

Prostatic small cell carcinoma is an aggressive subtype of prostate cancer that usually appears as a progression of the original adenocarcinoma. We describe here the WISH-PC2, a novel neuroendocrine xenograft of small cell carcinoma of the prostate. This xenograft was established from a poorly differentiated prostate adenocarcinoma and is serially transplanted in immune-compromised mice where it grows within the prostate, liver, and bone, inducing osteolytic lesions with foci of osteoblastic activity. It secretes to the mouse Chromogranin A and expresses prostate plasma carcinoma tumor antigen-1, six-transmembrane epithelial antigen of the prostate, and members of the E6-B receptor family. It does not express prostate-specific antigen, prostate stem cell antigen, prostate-specific membrane antigen, and androgen receptor, and it grows independently of androgen. Altogether, WISH-PC2 provides an unlimited source in which to study the involvement of neuroendocrine cells in the progression of prostatic adenocarcinoma and can serve as a novel model for the testing of new therapeutic strategies for prostatic small cell carcinoma.

Introduction

Primary SCCP is a relatively rare form of NE differentiation of PC. Nevertheless, it is clinically important because it is an extremely aggressive tumor with a very poor prognosis. NE differentiation of PC into SCCP is usually identified at the time of progression or recurrence of tumors that were originally classified as conventional adenocarcinoma of the prostate (1). This heterogeneity can be explained by divergent differentiation from multipotent stem cells (2, 3). Upon diagnosis of a small cell component, the clinical course is aggressive with common local and distant failure and a limited median survival duration of 9.8 months (2). It is therefore important to establish an experimental model of this tumor that will enable the testing and exploitation of potential therapeutic modalities.

The optimal treatment for SCCP has not been determined. The tumor often appears mixed with adenocarcinoma of the prostate, and it is usually treated with chemotherapeutic regimens designed for small cell carcinoma of the lung (1). The possible advantage of hormonal agents combined with chemotherapy remains unproven. Indeed, accelerated proliferation of the NE cells may represent an important step in the development of androgen-independent growth of PC driven by alternative growth signals. di Sant’agneuse (4) described the NE cells (also known as the endocrine-paracrine cells of the prostate) as intraepithelial regulatory cells displaying hybrid epithelial, neural, and endocrine characteristics. Although devoid of AR (5), the cells are capable of secreting alternative growth factors such as bombesin, serotonin, somatostatin, calcitonin, and parathyroid hormone-related protein (4–6). The prostatic NE cells express the c-erbB-2 growth factor receptors (7, 8). It was suggested that SCCP is composed of an enriched population of androgen-independent cells whose growth is sustained through alternate paracrine and autocrine pathways (6).

Only one model of human SCCP was reported thus far (9, 10). The WISH-PC2 line described here was derived from a PC patient, and expresses novel PC-specific molecular markers. This model should be extremely useful in studies aimed at the elucidation of critical aspects of the NE differentiation of PC, such as the regulatory mechanisms displayed by the NE cells and the interactions between the disseminated tumor and its various metastatic sites. In addition, the effect of various therapies on the primary tumor and on its disseminated form can be further evaluated.

Materials and Methods

Clinical History. The donor of the WISH-PC2 tumor tissue was a 67-year-old Caucasian male. He was diagnosed with T3N1M1 prostatic adenocarcinoma with a Gleason score of 8 (3 + 5). At the time of diagnosis, his serum PSA level was 53 ng/ml. Hormonal ablation was initiated with s.c. injection of 10.8 mg Goseroline every 12 weeks, resulting in a mild decline in serum PSA levels to a nadir of 40 ng/ml. A year later, while continuing this regimen, the patient complained of obstructive voiding symptoms and serum PSA levels rose to 65 ng/ml. Ultrasoundography demonstrated an enlarged obstructing prostate with a significant post-void residual urine volume. Anti-androgen (bicalutamide 50 mg/day) was added to the luteinizing hormone releasing hormone agonist. However, because of worsening of the obstructive voiding symptoms, the patient underwent a palliative transurethral resection of the prostate. The WISH-PC2 (Weizmann Institute Sheba Hospital Prostate Cancer) xenograft line was established from tissue samples obtained during this operation. The pathological examination revealed poorly differentiated carcinoma infiltrating the smooth muscle, with a typical NE differentiation (Fig. 1). As the patient’s disease continued to progress, the hormonal therapy was replaced with chemotherapy (cyclophosphamide, doxorubicin, and vinblastine). However, the patient’s condition continued to deteriorate, and he expired a few weeks after the initiation of cytotoxic therapy.

Establishment of the Xenograft. Animals and Surgical Procedures. Animals used were 4–10-week-old SCID (c-b 17Icr Beige or NOD) and nude (BALB/c nu/nu) mice obtained from the pathogen-free animal breeding facilities of the Weizmann Institute of Science. All of the surgical procedures were performed under ketamine + xylazine general anesthesia (127.5 and 4.5 mg/kg, respectively), except for the insertion of s.c. testosterone pellets and s.c. tumor pieces, which was performed under local anesthesia with xylocaine 10% spray (Astra Sweden). The original surgical samples of the tumor were placed on ice, minced into 3–5-mm pieces, and implanted s.c. into SCID mice. After an initial latency period of 4 months, tumor growth was noticed in 30% of the mice. Therefore the tumor was serially passaged (upon submission of the manu-
hepatic lobes using a 27-gauge needle. With 100 mL cell suspensions (20 mL tumors. Doubling time of the tumor growth was calculated during the logarithmic growth phase of subcutaneously growing tumors.

Tumor size was determined by caliper measurements of length, width, and depth, and the tumor volume (mm$^3$) was approximated using the formula: length $\times$ width $\times$ depth $\times 0.5236$ (11). Doubling time of the tumor growth was calculated during the logarithmic growth phase of subcutaneously growing tumors.

**Injection of Tumors to Various Organs.**

**Orthotopic Injection.** Tumor cell suspensions (20 mL) were injected into the dorsal prostatic lobes through a midline lower abdominal incision as described by Stephenson et al. (12). A well-localized bleb within the injection site was considered to indicate technically satisfactory injection.

**Intrahepatic Injection.** A midline abdominal incision was performed to expose the liver. Tumor cell suspensions (10–20 mL) were injected into both hepatic lobes using a 27-gauge needle.

**s.c. Injection.** Mixtures of tumor cell suspensions in various concentrations with 100 mL of Matrigel basement membrane matrix solution (Becton Dickinson) were injected s.c. using a 27-gauge needle.

**Preparation of Single-Cell Suspension.** The xenografted tumor tissue was harvested under sterile conditions and placed immediately in cooled HBSS, recommended by the manufacturer (DAKO Denmark).

**Immunohistochemistry.** Immunohistochemistry was performed on sections from formalin-fixed, paraffin-embedded blocks as described previously (14). Antibodies used included mouse monoclonal antibodies to PAP, PSA, NSE, chromogranin and synaptophysin, obtained from DAKO Corp., Carpinteria, CA. Antibodies to human AR were purchased from Innovex Biosciences, USA. Antibodies to PSCA was a gift from Dr. Robert Reiter (University of California at Los Angeles). After incubation with monoclonal antibodies, slides were incubated sequentially with peroxidase-conjugated rabbit antihuman immunoglobulins, and peroxidase-conjugated swine antirabbit immunoglobulins. Antibody localization was visualized with the diaminobenzidene reaction. Negative controls consisted of substitution of the primary antibody with an isotype matched non-cross-reacting antibody of irrelevant specificity.

Immunohistochemical techniques combined with image analysis were used to detect the presence of bcl-2, P glycoprotein (MDR1), and p53. An experienced commercial laboratory performed these pathological evaluations as well as determination of DNA ploidity and proliferation index of the tumor samples (Quantitative Diagnostic Laboratories).

Serum PSA levels were determined by Immulite Third generation PSA kit (Diagnostic Products Corp., Los Angeles, CA). Plasma chromogranin-A levels were quantified using an ELISA kit as recommended by the manufacturer (DAKO Denmark).
Results and Discussion

NE differentiation of PC is associated with poor prognosis and resistance to anti-androgen therapy (5, 15). NE differentiation can take several forms, including NE SCCP, carcinoid-like tumor or, most commonly, focal NE differentiation in conventional prostatic adenocarcinoma. In this study, we established a xenograft model that could recapitulate many of the clinical characteristics of SCCP. As such, it enables phenotypic characterization and the development and evaluation of possible therapeutic modalities.

Growth Pattern of the WISH-PC2 Xenograft. The patient from whom the WISH-PC2 xenograft was established was initially diagnosed with conventional high-grade adenocarcinoma of the prostate (Fig. 1). The tumor converted to SCCP in parallel to an expedient clinical course of progression, emphasizing the linkage between the two subtypes of the prostatic tumor (1, 2, 16). Indeed, in the first generation, 20% of the mice into which the tumor pieces were implanted had elevated serum PSA levels. The WISH-PC2 xenograft was established from a tumor-bearing mouse that did not exhibit elevated serum PSA levels. We determined that the WISH-PC2 cells are of human origin and did not result from an overgrowth of the explants by murine cells (17). The xenograft is stained with anti-HLA-A,B,C antibodies (data not shown). In addition, the tumor does not express the B-cell CD19, CD20, and CD22 differentiation antigens (data not shown), excluding the possibility of being an overgrowth of dormant EBV-transformed human B cells (18). The xenografted carcinoma is highly cognate in its gross histological appearance to the donor’s surgical specimen, and it shares the expression of NE tumor markers (Fig. 1): chromogranin A, NSE, and synaptophysin. Notably, chromogranin A is also secreted into the plasma of WISH-PC2-bearing mice, and the plasma concentration of chromogranin A is correlated to the size of the xenograft (data not shown). Hence, chromogranin A can serve as a secreted tumor marker to monitor the growth of this NE SCCP xenograft.

The WISH-PC2 xenograft grows relatively rapidly (Fig. 2) and with a high take rate (visible tumor growth is evident in 90–100% of the animals). Upon s.c. injection of tumor cells or implantation of tumor tissue, growth can be detected within 2–3 weeks. In the presence of Matrigel, the doubling time of the tumor after the s.c. implantation of \(3 \times 10^6\) cells, is 11 or 13.5 days for a tumor growing in the presence or absence of androgen, respectively, and 15 and 18 days, respectively, for a tumor that arises from s.c. implantation of tissue (70 mg) with and without androgen supplementation. This pattern of androgen-responsive growth most probably reflects an indirect effect of androgen, inasmuch as the tumor cells do not express ARs as expected for SCCP (1, 3). Because the WISH-PC2 xenograft has been originated from a mixed-type tumor, there is a possibility that it is a mixture of SCCP with some residual adenocarcinoma cells that are hormone-responsive and expand in the presence of androgen. To test this, we stained the WISH-PC2 tissue that grew for more than 80 days in the presence of a continuous supply of testosterone with anti-PSA and anti-AR antibodies. The results did not reveal any staining above background of these tumors. Moreover, RT-PCR analysis of RNA derived from WISH-PC2 that grew in the absence or presence of androgen was completely negative for PSA and AR (data not shown). Similarly, no PSA could be detected in the sera of mice bearing WISH-PC2, regardless of whether they were hormone-supplemented or not. All these data argue against the possibility that the enhanced growth observed in the presence of androgen (Fig. 2) is attributable to residual androgen-responsive adenocarcinoma cells. Apparently, the serial transfer of WISH-PC2 in the absence of an external source of testosterone in the first few generations provided a selective advantage to the SCCP component over the adenocarcinoma. Androgen was demonstrated to have indirect effects on PC via up-regulation of surrounding stromal vascular endothelial growth factor production and angiogenesis (19). The high growth rate is also reflected by the high proliferation index, depicted by staining with the Ki-67 antibody, and directed at a nuclear antigen expressed in proliferating cells (data not shown).

Growth of WISH-PC2 in Various Organs. To establish a valid model in which to test various therapeutic strategies, we developed a xenograft model using WISH-PC2 cells that would closely model human SCCP and its metastasis. The ability of the WISH-PC2 xenograft to grow orthotopically within the murine prostate (Fig. 3) provides such a model. Interestingly, orthotopically transplanted human small cell lung carcinoma displays a different chemosensitivity pattern compared with the s.c. transplanted model (20).

One of the typical clinical features of SCCP, in contrast to adenocarcinoma of the prostate, is its tendency to develop visceral metastasis. The most frequent metastatic sites of SCCP are the bones (55%), regional and distant lymph nodes (52%), and the liver (48%; Ref. 2). Occasionally, liver, lung, and lymph node metastasis can be recovered after s.c. implantation of WISH-PC2. Interestingly, a high incidence of metastasis are found after direct intrahepatic injection of the xenograft cells, especially in surgical wound sites such as that in mice which have undergone bilateral transabdominal orchiectomy for an-
of the bony plaque, lytic changes are formed by tumor cells (WISH-PC2 cells were injected is distorted by tumor cells (black arrows). Note a bony “plaque” surrounded by abundant osteogenitor cells and osteoblasts (white arrows). Tumor marker indicating the location of the right and left legs; M, tumor. F, plain radiography to the same mouse, demonstrating lytic distraction of the bone only at the site of tumor injection (white arrows) and not in the contralateral control site (TI, site of the tail injection of the radioisotope). UB, urinary bladder; M, marker indicating the location of the right and left legs; T, tumor.). G, histological view of the right proximal tibia of the mouse that was injected with PBS demonstrating normal bone architecture, including epithysis, bone marrow cells, bone trabeculi. (H&E staining; original magnification 400). H, bone architecture in the left proximal tibia of the mouse into which the WISH-PC2 cells were injected is distorted by tumor cells (T). Note a bony “plaque” surrounded by abundant osteogenitor cells and osteoblasts (black arrows). On the right upper side of the bony plaque, lytic changes are formed by tumor cells (open arrow; H&E staining; original magnification 200).

**Malignant Phenotype of the WISH-PC2 Cells.** Next we tested WISH-PC2 cells for the expression of several prostate specific markers. Table 1 lists various molecular markers whose expression was evaluated on WISH-PC2. The xenograft does not express PSA, PSMA, PSCA, or PAP. Nevertheless, its prostatic origin is supported by the following molecular markers: (a) expression of cytokeratin 8 and 18 common to PC and prostatic secretory cells (3); (b) expression of PCTA-1, which is a surface marker of PC and its precursor, prostatic intraepithelial neoplasia, but is not found on normal prostate or benign prostatic hyperplasia (22); and (c) expression of the STEAP. This recently described surface marker (23) is highly expressed at all stages of PC and does not seem to be modulated by androgen. Although STEAP is also expressed in multiple cancer cell lines, its expression in normal human tissues is restricted to the prostate and bladder.

**Immunostaining of WISH-PC2 xenograft sections demonstrated that the tumor expresses adverse pathological features reflecting the aggressive nature of this tumor.** These include aneuploid DNA content, bcl-2 protein, and mutated p53 (Table 1). Although previously prostate NE cells were reported not to express the antiapoptotic bcl-2 marker (24), these cells were terminally differentiated, nonproliferating cells. The WISH-PC2 cells represent malignant prostatic NE cells.

**Table 1 Phenotypic features of WISH-PC2**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA ploidy</td>
<td>Aneuploid</td>
</tr>
<tr>
<td>Proliferative Index (Ki-67)</td>
<td>High</td>
</tr>
<tr>
<td>Bcl-2a</td>
<td>Positive</td>
</tr>
<tr>
<td>Mutated p53a</td>
<td>Positive</td>
</tr>
<tr>
<td>MDR1 gene producta</td>
<td>Negative</td>
</tr>
<tr>
<td>PSAa,b</td>
<td>Negative</td>
</tr>
<tr>
<td>PSCAb</td>
<td>Negative</td>
</tr>
<tr>
<td>PSAAb</td>
<td>Negative</td>
</tr>
<tr>
<td>PAPa</td>
<td>Negative</td>
</tr>
<tr>
<td>ARa,b</td>
<td>Negative</td>
</tr>
<tr>
<td>STEAPa</td>
<td>Positive</td>
</tr>
<tr>
<td>PCTA-1/galactin-8c</td>
<td>Positive</td>
</tr>
<tr>
<td>Cytokeratin 8b</td>
<td>Positive</td>
</tr>
<tr>
<td>Cytokeratin 18b</td>
<td>Positive</td>
</tr>
<tr>
<td>Chromogranin Aa,d</td>
<td>Positive</td>
</tr>
<tr>
<td>NSEa</td>
<td>Positive</td>
</tr>
<tr>
<td>Synaptophysinab</td>
<td>Positive</td>
</tr>
<tr>
<td>Her-2neua</td>
<td>Positive</td>
</tr>
<tr>
<td>Her-3neua</td>
<td>Positive</td>
</tr>
<tr>
<td>Her-4neua</td>
<td>Positive</td>
</tr>
<tr>
<td>MHC class-Ia</td>
<td>Positive</td>
</tr>
</tbody>
</table>

a Determined by immunohistochemistry.
b Determined by RT-PCR.
c Determined by Western blot analysis.
d Determined by ELISA of murine host plasma.
e Determined by FACS analysis.
It is therefore intriguing to speculate that both bcl-2 and defective p53 allow these AR-negative cells to survive and overtake the adenocarcinoma cells, which undergo apoptosis in response to androgen deprivation and, potentially, chemotherapy.

FACS analysis using monoclonal antibodies against the epidermal growth factor receptor erb-B family revealed that WISH-PC2 expresses erb-B2, erb-B3, and erb-B4 on its surface. The expression of these growth factor receptors was stable throughout all of the passages of the tumor (data not shown). Iwamura et al. (7) demonstrated immunostaining of c-erb-B2 on prostatic NE cells using polyclonal antibodies. To the best of our knowledge, no data have been reported to date concerning the coexpression of erb-B3 and/or erb-B4 with erb-B2 on prostatic NE cells, a combination that is necessary for the binding of Neuregulin ligands to these receptors and for their activation (13). The presence of the erb-B set of receptors may provide an alternative pathway of growth signaling for the androgen-independent proliferation of these cells, either directly or by regulating NE peptides that function in an autocrine or paracrine manner.

Potential Application of the WISH-PC2 in Therapeutic Models. Prostatic small cell carcinoma is a notoriously aggressive malignancy with a very poor prognosis (21). No effective treatment for SCCP has been established, most probably because of the limited patient population and the aggressiveness of the disease. WISH-PC2 provides an in vivo model for the evaluation of different possible therapeutic strategies for SCCP with an inherent plasma tumor marker (chromogranin A).

The issue of whether castration is a justified treatment for SCCP is still unresolved. The progression from adenocarcinoma of the prostate to SCCP usually appears after castration (1). However, based on the evidence that most SCCP are mixed with adenocarcinoma of the prostate, the common practice is to combine hormonal and cytotoxic therapy (21, 25). The fact that androgen supplements somewhat increase WISH-PC2 tumor growth (Fig. 2) suggests that androgens may enhance the growth of the AR-negative xenograft, probably via an indirect effect on the surrounding stroma (19). It is therefore possible that in the case of SCCP, especially those present as mixed histology (adenocarcinoma and small cell elements), hormonal manipulation may slow tumor progression.

The WISH-PC2 model can serve as a useful model for testing established and novel cytotoxic drugs. Targeted drug delivery to the various anatomical sites of visceral distribution of SCCP, such as liver or bones, may be readily tested in this model system. The WISH-PC2 xenograft, lacking the p-170 multi-drug efflux pump (MDR1 Table 1), that mediates the MDR phenotype, is therefore susceptible to chemotherapy. It has still to be demonstrated however, whether the WISH-PC2, expressing mutated p53 and bcl-2 are susceptible to apoptosis-inducing drugs.

In conclusion, the WISH-PC2 SCCP xenograft is an excellent source for NE prostatic cells and their factors in studies of intercellular interactions that take place during PC progression. Moreover, this novel human xenograft can serve as a model for the exploitation of new therapeutic modalities on this aggressive variant of PC.

Acknowledgments

We are indebted to Dr. A. Jakobovits for PCR analysis of prostate markers, to K. Kaufman for technical assistance, to D. Nathan for pathological procedures, to Drs. A. Schwartz and E. Goshen for bone scanning, to Dr. I. Aizenberg for radiological imaging, and to Dr. S. Schwarzbaum for critical review of the manuscript.

References

WISH-PC2: A Unique Xenograft Model of Human Prostatic Small Cell Carcinoma


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/23/6563

Cited articles
This article cites 23 articles, 5 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/23/6563.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/60/23/6563.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.