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Review

Small-cell neuroendocrine carcinoma of the prostate: are heterotransplants a better experimental model?

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Abstract

Small-cell neuroendocrine carcinoma of the prostate (SCNCP) is an uncommon type of prostate cancer. However, it is of clinical importance because it is one of the most aggressive tumors of the prostate with a very poor prognosis. There exist few artificially cultured tumor cell lines to study SCNCP. Then, another approach to that study consists in the use of fresh tumor tissue obtained from patients and its heterotransplantation into host mice. The purpose of this review is to integrate data from more than 20 years of heterotransplantation research in the study of small-cell neuroendocrine carcinoma of the prostate (SCNCP). Heterotransplantation has provided data regarding the histopathology, karyotype, DNA content, cell cycle frequency, tumor markers, androgen receptor expression, metastasis and take rate of this prostate disease. When possible, comparisons between original *in situ* specimens removed from patients and heterotransplanted tissue from host mice have been made. There are advantages, as well as limitations, that have been identified for SCNCP heterotransplants versus xenotransplantation of cultured cells. Overall, heterotransplanted tumors are better than conventional tumor xenografts at retaining tumor morphology, pathology, secretory activity and expression of tumor markers of the patient's original specimen. Furthermore, heterotransplanted tissue preserves the three-dimensional tumor architecture of the prostate to maintain critical stromal-epithelial cell interactions.

1 Introduction

Small-cell neuroendocrine carcinoma of the prostate (SCNCP) is a rare form of neuroendocrine differentiation of prostate cancer comprising 0.5%–2% of all prostate carcinomas [1]. As an aggressive type of cancer, SCNCP is usually diagnosed at an advanced stage [2].

Although devoid of androgen receptors (AR), tumor cells are capable of secreting growth factors such as bombesin, serotonin, somatostatin and calcitonin. Unfortunately, SCNCP has a tendency to metastasize to bones, regional and distant lymph nodes, and the liver. Currently, treatment of SCNCP includes radiation therapy, chemotherapy and surgery.

In the field of preclinical experimental therapy, there is a great need for tumor models relevant to human prostate diseases. The use of mouse xenograft models to represent preclinical tumors has become more widespread, as this model is reasonably inexpensive, can provide rapid experimental data, is largely free of regulatory constraints and can be used with many

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different tumor types [3, 4]. Artificially cultured SCNCP tumor cell lines, SO-MI and LnCaP, can be implanted in immunosuppressed mice. There have been an increasing number of studies using this approach. Despite the increased use of these xenograft models, there are disadvantages to this approach [5]. First, not all tumor cell subpopulations consistently maintain the genetic aberrations that enable the tumor cells to be maintained in cell culture. Second, *in vitro* culturing over several years has the potential to select for certain cell subtypes that may or may not be relevant to prostate cancer, and therefore may no longer be representative of the original tumor [5]. Third, the generation of cell lines for *in vitro* culturing currently includes only a limited number of tumor biopsies. Fourth, the phenotype of epithelial cells cultured from prostate tumors largely suggests that they are luminal in origin and not derived from basal cells. Taken together, these aspects of *in vitro* culturing suggest that the resulting subsets of tumor cells generated may not accurately represent the genetic diversity of the disease.

In gene expression studies of small-cell neuro-endocrine carcinoma of the lung, some genes have been shown to undergo irreversible changes in expression after the cells are cultured *in vitro*. Furthermore, expression patterns for a large number of genes were not restored when the derivative cell line was returned to growth *in vivo* as a xenograft [6]. In general, gene expression data have shown that primary biopsy heterotransplants into mice more closely resemble the original patient tumor than derived cell lines and associated xenografts. These results suggest that tumor cells acclimate to cell culture conditions and do not completely regain the original gene expression profile characteristic of small-cell neuroendocrine carcinoma of the lung in humans. Expression changes also typically occurred in a large number of critically important cancer-signaling pathways directly related to targeted therapies, epithelial–stromal interactions and developmental signaling [6]. Additional evidence has shown that in the absence of three-dimensional tumor–stromal interactions, cultured cell lines exhibit a morphology that is distinctly different from the tumor of origin, with xenografts derived from cultured cell lines exhibiting a more homogeneous, undifferentiated histology [7, 8].

The cell types contained in the prostate include luminal and basal epithelial cells, neuroendocrine cells, smooth muscle cells, fibroblasts, nerves and basement membranes that interact to coordinate function in the prostate (Figure 1, [9]). Prostate stromal cells have been shown to have an important role in the development of SCNCP tumors, and often supply tumor cells with the cell signals and nutrients they need [10]. It is important to note that the necessary interactions between cell types of the prostate are not completely reconstituted in xenografts, as proteins from one species cannot always interact with their counterparts in the host. However, when co-implantation of human tumor cells with human stromal cells is performed, the tumors generally recapitulate the human disease. The latter approach has not been taken in SCNCP xenografts. SCNCP tumor models are further limited by the fact that in addition to humans, only primates and dogs develop prostate tumorigenesis with age [11]. Unfortunately, both of these animal models are very expensive to maintain and are subject to stringent regulatory constraints. Another possible tumor model involves rodents; however, the extrapolation of the results obtained with this tumor model to human disease is limited by its non-human origin. Alternatively, the prostate heterotransplant
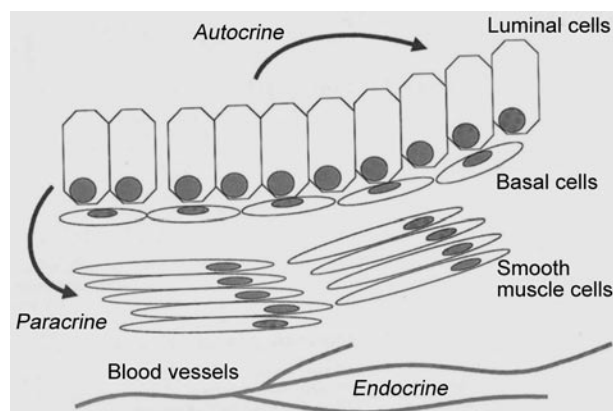

Figure 1. Cells of different histologic types compose the prostate gland. Basal cells, luminal cells, small neuroendocrine cells, smooth muscle cells, fibroblasts, vascular cells, nerve cells and basement membrane plus tissue matrix compose the prostate gland. Hormones like testosterone utilize endocrine pathways to affect the prostate microenvironment. Androgen-mediated events use either autocrine signaling pathways, in which the cell secretes peptide growth factors to influence its own growth (e.g., luminal cells to luminal cells), or paracrine pathways, in which the cell elaborates peptide growth factors to affect neighboring cells of a different histologic type. (Reproduced from Prostatic Diseases [9] with permission).

model includes all the cell types present during the development of the SCNCP, and the architecture of the gland is preserved. The spatial relationship between the stromal and epithelial cell compartments is also maintained, along with the necessary vasculature, as the human cells are able to proliferate and differentiate [11].

To evaluate the SCNCP heterotransplant model, it is necessary to know how well the heterotransplanted tumor can maintain the anatomical architecture and function of the original tumor collected from the patient. A key aspect of the heterotransplant model is that the human biopsy is inserted in a living host from a different species, and it is the interactions between these two microenvironments that have been studied for the past 20 years. It is in this body of work that human prostate tumor heterotransplantation has defined how human tumors maintain their integrity, and yet change as a result of their interactions with host cells. The interactions that take place at this human-mouse interface of the heterotranplanted model are the focus of this review.

Note: In the text, the standard heterotransplant implantation site for the patient's tumor is considered to be the subcutaneous (sc) space of the host mice. When other implantation sites have been used, such as the subrenal capsule or the prostate itself, the location will be specifically cited.

2 Human SCNCP heterotransplanted into immuno-suppressed mice—similarities with the patient tumor of origin

2.1 Cytological and histological analysis

The original heterotransplantation of a patient's SCNCP tumor has been shown to be histologically similar to the original surgical specimen collected independent of the strain of mice used (that is, athymic balb/c nude or CB17 non-obese diabetic-severely combined immunodepressed (scid) mice, the use of Matrigel® (Becton-Dickinson, Franklin Lakes, NJ, USA), or testosterone (T) supplementation [12–15]. Matrigel is an extracellular matrix gel composed of type IV procollagen, laminin and heparan sulfate proteoglycan obtained from the Engelbreth-Holm-Schwarm mouse sarcoma first described by Kleinman *et al.* [16]. For example, four reports have described SCNCP heterotransplants serially passaged in mice. The original report described a heterotransplant that did not become anaplastic and the basic pathology

remained unchanged after serial transplantation [11]. The second study by van Haaften-Day *et al.* [12], involved the heterotransplantation of the tumor, UCRU-PR2, derived from a poorly differentiated carcinoma. The undifferentiated SCNCP retained the characteristics of the patient's original tumor as a neuroendocrine carcinoma that presented occasional small neurosecretory granules. Cell suspensions from this heterotransplant at passage seven were implanted intramuscularly, intraperitoneally and in the kidney capsule. At all of these sites tumors developed, which were histologically identical as small-cell undifferentiated carcinomas. However, tumor fragments implanted in the liver and spleen failed to develop tumors as monitored between passages second and fifth [17]. The third heterotransplant, WISP-PC2, another poorly differentiated carcinoma, was also similar to the original patient specimen and was maintained through 12 passages. The heterotransplanted tumor stained positive for human HLA-A, -B and -C, and did not express the B-cell differentiation antigens CD19, CD20 and CD22 [13]. These results indicate that an overgrowth of the heterotransplant by murine cells did not occur. In 2002, True *et al.* [14] heterotransplanted a pelvic and omentum lymph node metastasis that generated the tumor cell line, LuCaP-49. The LuCaP-49 tumor has been serially passaged for 5 years and over the entire period it has maintained the original phenotype.

2.2 Antiangiogenesis drug testing

The WISP-PC2 heterotransplanted tumor has been used as a model of SCNCP to investigate the antitumor activity of the collagen type I synthesis inhibitor, halofuginone [18]. The treatment with halofuginone caused a remarkable reduction in tumor volume when implanted sc or orthotopically that was dose-dependent and independent of the route of administration, oral or intraperitoneal. Halofuginone caused an inhibition of WISP-PC2 cells invasion into the Matrigel plug, reduced collagen content and EC number in the tumor resulting in an increase in tumor cell apoptosis/necrosis ratio [18].

2.3 Karyotype, DNA content and cell cycle frequency

Four SCNCP heterotransplanted tumors had their DNA content determined during their first passage. The heterotransplanted tumor, UCRU-PR2, obtained by van Haaften-Day *et al.* [12] had a diploid DNA content similar to the original carcinoma tumor.

However, starting with the seventh passage, 50% of the heterotransplants developed an aneuploid population in addition to the original diploid population [17]. Pinthus *et al.* [13] determined the DNA content of the heterotransplanted tumor, WISP-PC2, to be aneuploid, whereas another heterotransplant had converted from a hypodiploid to hypertriploid DNA content, and a third heterotransplant maintained a hypertriploid DNA content profile.

An analysis of cell cycle distribution for the heterotransplanted tumors was also informative. For example, the patient tumor from which the UCRU-PR2 heterotransplant was derived had a distribution among the three cell cycle phases of 81.6%, 11.4% and 6.9% for the G_0/G_1-phase, S-phase and G_2/M-phase, respectively. The third passage of the UCRU-PR2 also had its cell cycle analyzed and the heterotransplant was nearly identical in cell cycle frequency to the original tumor with a distribution of 83.7%, 10.1% and 6.0% in the G_0/G_1-phase, S-phase and G_2/M-phase, respectively [12]. Similar results were found in a separate study of the same heterotransplant for the second and fourth heterotransplanted passages. The cell cycle distribution included 81.4% ± 2.3% and 79.4% ± 3.2% in the G_0/G_1-phase; 10.0% ± 0.7% and 10.7 ± 0% in the S-phase, and 8.6% ± 0.7% and 10.0% ± 3.2% in the G_2/M-phase, respectively [19]. For the same heterotransplanted SCNCP tumor at its fifth passage, the modal chromosome number was 43 with a range of 42–45 chromosomes. Karyotyping of heterotranplanted tumors at different passages has shown the remarkable chromosome stability that is maintained despite repeated serial heterotransplantations [19]. In contrast, the LuCaP-49 heterotransplant includes chromosomal aberrations that were detected by comparative genomic hybridization [20]. This heterotransplant originated from a patient that was not treated with androgen withdrawal before removal of the biopsy.

2.4 Marker expression and functional activity

Analysis of protein markers expressed by heterotransplants has also been performed. Carcinogenic embryonic antigen (CEA) and epithelial membrane antigen (EMA) were detected in the original patient tumor specimen collected by van Haaften-Day *et al.* [12], as well as in the first passage of the corresponding UCRU-PR2 heterotransplant. Strong expression of CEA was maintained along with focal positive staining for EMA through passages 2 and 3 of UCRU-PR2. Neuroendocrine molecules such as adrenocorticotropic hormone, β-endorfin and its acetylated form, NaCEP, and somatostatin were also detected in the UCRU-PR2 heterotransplant [21]. For neuro-specific γ-γ enolase (NSE), it was expressed in the serially passaged tumor heterotransplants but not in the original patient tumor specimen [12]. Cell suspensions from the seventh passage of UCRU-PR2 were also implanted intramuscularly, intraperitoneally and in the kidney capsule. The majority of heterotransplants in each implantation site had the same staining pattern as the original tumor specimen. All of the tumors expressed EMA and NSE, yet were negative for prostate acid phosphatase (PAP), prostate serum antigen (PSA), and keratin (Table 1). Staining for CEA exhibited variable intensity between the samples [17]. The original patient tumor specimen that resulted in the WISP-PC2 heterotransplant also exhibited strong staining for the expression of neuroendocrine tumor markers such as chromogranin A, NSE and synaptophysin, yet did not stain for PSA [13]. The secretion of chromogranin A into the circulation of tumor-bearing mice and as a result its plasma concentration has been shown

Table 1. Human small cell neuroendocrine carcinoma of the prostate heterotransplants.

Tumor model	Origin	Prior treatment of patient	Androgen status	AR	PAP	PSA	Lag phase (months)	Td (days)	Reference
UCRU-Pr2	TURP	Bilateral orchidectomy	−	−			4.0		[12]
WISH-PC2	TURP	Hormonal ablation anti-androgen	Indirect effect	−				15–18[a]	[13]
LuCaP-49	Pelvic LN and omentum	Radiotherapy and radical cysto-prostatectomy	−	−	−	−	7.7 ± 2.4[b] 9.0 ± 1.7[c]		[14]

Abbreviations: AR, androgen receptor; LN, lymph nodes; PAP, prostate acid phosphate; PSA, prostate serum antigen; Td, tumor doubling time; TURP, transurethral resection of the prostate.
[a]Coimplanted with Matrigel® and mice supplemented with testosterone pellet; [b]intact mice; [c]orchiectomized mice.

to correlate with heterotransplant tumor size. The immunophenotype of the LuCaP-49 heterotransplant was also found to be essentially identical to that of the SCNCP component of the primary tumor from which it was derived. In both samples, positive staining for the expression of the adhesion molecule human natural killer 1, or LEU7 (CD57), normally associated with neurons, was identified. Positive staining for LMW keratin, MIBI/Ki67, NSE and synaptophysin was also detected. In contrast, negative staining for hyaluronic acid receptor (CD44), high-molecular-weight keratin, PSA, PSAP and vimentin was observed for both samples [14]. Given that the morphological and immunohistochemical data showed that LuCaP-49 was nearly identical to the primary tumor from which it was derived, Clegg *et al.* [22] constructed a cDNA library and studied NE gene expression in the prostate. Expression of achaete–scute complex homolog-like 1 (ASCL1), internexin neuronal intermediate filament protein-α (INA) and synaptic vesicle protein 2B homolog (SV2B) has been identified as potential molecular markers for SCNCP, and were detected in LuCaP-49, but not in other prostate cancer cell lines and xenografts tested.

2.5 Androgen-independent growth

Three of the successfully and serially transplanted SCNCP tumors, UCRU-PR2, WISH-PC2 and LuCaP-49, do not express AR unlike their respective original patient specimens [12–14] (Figure 1, [9]). Furthermore, the UCRU-PR2 heterotransplant does not express estrogen receptor [12] (Table 1). Corey *et al.* [23] hypothesized that 17-β-estradiol (E) may have an inhibitory effect on prostate carcinoma in general, and on SCNCP in particular. The LuCaP-49 heterotransplant model has the advantage that it does not express AR, and correspondingly is androgen insensitive. This model has a take rate of 40% (6/15) in intact females and 60% (9/15) in ovariectomized females. Additionally, a delay in tumor growth was observed for non-ovariectomized females versus ovariectomized females, supporting the hypothesis that E can inhibit prostate cancer independent of androgen suppression. Similarly, a delay in tumor growth was observed for LuCaP-49 heterotransplants in orchiectomized males supplemented with E compared with non-treated controls [24].

2.6 Metastasis

Only one report has described the implantation of patient tumor specimens with Matrigel (Becton-Dickinson), to be associated with the liver, lung and lymph node metastases [13]. In contrast, an endovenous administration of harvested cells from a UCRU-PR2 heterotransplant did not result in experimental metastases in the lung [17]. Alternatively, it has been reported that WISH-PC2 and WH-4A heterotransplant-bearing mice developed metastases in their adrenal glands as a result of irradiation of the primary tumor [25]. From a clinical perspective, these data would suggest that radiation to treat SCNCP may promote tumor metastasis. Thereby, patients whose prostate biopsies show a predominance of SCNCP may require systemic therapy in combination with radiotherapy to prevent the development of metastases [25].

2.7 Heterotransplantation take rate

Two studies have described the heterotransplantation of SCNCP tumors into host mice, and both studies achieved a take rate of 100%. van Haaften-Day *et al.* [12] used athymic nude Balb/c mice that did not receive T-supplements for their heterotransplant model of a hormone-independent and undifferentiated SCNCP tumor. Alternatively, Pinthus *et al.* [13] heterotransplanted a poorly differentiated SCNCP carcinoma with Matrigel (Becton-Dickinson), into scid mice supplemented with T-pellets.

3 Experimental limitations in the use of human heterotransplants

Access to, and availability of, human tumor specimens requires a coordinated team of highly trained clinical professionals including oncologists, surgeons, pathologists, internists, nurses, social workers and paralegals. In addition, these professionals have to coordinate their efforts with a team of researchers and laboratory assistants who maintain a database of samples and data to ensure the proper storage, analysis and implantation of patient specimens into immunosuppressed mice. Communication between these two groups is one of the challenges in performing human heterotransplant experiments.

A second challenge for human prostate heterotransplant experiments is the inevitable contamination of samples by host cells. For example, during the serial passaging of primarily stromal cells, host vasculature and infiltration of host inflammatory cells are present and are not able to be easily separated. Therefore, *in situ* hybridization using human probes and immunocytochemistry using

antibodies against human epitopes help identify human versus mouse tissue in heterotransplants in spite of highly conserved sequences shared between human and mouse homologs [26].

4   Experimental advantages of human heterotransplant models

When patient tumor specimens are initially heterotransplanted into host mice, a majority of them maintains the biology of the original patient tumor. For example, the heterotransplanted tumor does not become anaplastic and the basic pathology remains unchanged even after serial passaging. Histology of the prostate gland is also well preserved with epithelial cells lining the gland adjacent to the stroma. Preservation of stromal cell-epithelial cell interactions from the original primary tumor is important.

Regarding the heterotransplanted tumor, like the original patient tumor, has active secretory epithelial cells, which express PSA and PAP. Similarly, SCNCP heterotransplants express CEA, EMA and NSE. However, heterotransplants have been shown to lose AR expression that was originally detected in the patient specimens before implantation. In terms of cell cycle distribution, even after serial transplantation, the heterotransplanted SCNCP tumor cells have a similar cell cycle distribution as the original patient specimens. Taken together, these data indicate that the heterotransplant model is largely representative of SCNCP disease, and this model is qualitatively superior to models that use human tumor cells collected after *in vivo* tumor xenograft induction.

The human tumor heterotransplant model provides an opportunity to study the expression of human genes within a primarily *in vivo* human context, and within the particular human prostate tissue environment that strongly influences gene expression and cell growth. The behavior of cancer cells can be studied, especially in response to experimental therapies. Proteomic and pharmacogenomic data can also be collected. As the heterotransplant model facilitates the growth of human SCNCP tumors, new antiangiogenic and chemotherapeutic agents can be evaluated for their antitumor potential. Also, new therapeutic regimens for the treatment of this disease can be tested. In all of these applications, the ability to cryopreserve part of the patient specimens before heterotransplantation remarkably increases the flexibility of the heterotransplant model.

5   Conclusions

The majority of heterotransplanted tumors in host mice have been shown to retain the biological properties that characterize the original human primary tumor specimen collected. These properties include tumor morphology, pathology, karyotype, secretory activity and the expression of important tumor markers. Preservation of the original three-dimensional tumor architecture is necessary for maintaining stromal cell-epithelial cell interactions and needed vasculature. Heterotransplantation of human prostate tumors provides a model that is qualitatively superior to a xenograft model that uses tumor cells cultured *in vitro* and results in a tumor dominated by a murine-based stromal and vascular environment. The heterotransplant model has been shown to provide long-term growth for a variety of human prostatic tumors, which facilitates the investigation of new agents and therapeutic protocols for the treatment of SCNCP. Furthermore, the human tumor heterotransplant model has the potential to provide proteomic and pharmacogenomic data relevant to the *in vivo* situation. In summary, implantation of a human tumor specimen into a mouse host provides a long-term, propagatable, *in vivo* heterotransplant model of SCNCP, and represents an evident advance in the ability to develop treatment strategies for prostate diseases.

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