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INVITED RESEARCH HIGHLIGHT

Prostate Cancer

Next generation patient-derived prostate cancer xenograft models

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There is a critical need for more effective therapeutic approaches for prostate cancer. Research in this area, however, has been seriously hampered by a lack of clinically relevant, experimental *in vivo* models of the disease. This review particularly focuses on the development of prostate cancer xenograft models based on subrenal capsule grafting of patients' tumor tissue into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. This technique allows successful development of transplantable, patient-derived cancer tissue xenograft lines not only from aggressive metastatic, but also from localized prostate cancer tissues. The xenografts have been found to retain key biological properties of the original malignancies, including histopathological and molecular characteristics, tumor heterogeneity, response to androgen ablation and metastatic ability. As such, they are highly clinically relevant and provide valuable tools for studies of prostate cancer progression at cellular and molecular levels, drug screening for personalized cancer therapy and preclinical drug efficacy testing; especially when a panel of models is used to cover a broader spectrum of the disease. These xenograft models could therefore be viewed as next-generation models of prostate cancer.

INTRODUCTION

Prostate cancer is the most commonly diagnosed noncutaneous cancer and second leading cause of cancer-related death of North American males.[1] The disease is at present incurable once it has metastasized, and most deaths from this disease are due to metastases that are highly resistant to current conventional therapies. Prostate cancer is considered a multifocal disease that generally consists of a dominant cancer and one or more concurrent cancers of smaller volume with different histological features covering a wide spectrum of biological behavior.[2–5] The biological and genetic heterogeneity of the cancers suggests that the foci arise from different clones.[6–9] The development of localized prostate cancer and the diversification and malignant progression to metastatic and castration-resistant forms are highly complex processes and thought to result from (i) changes in the expression of specific genes particularly in epithelial prostatic cells and (ii) alterations in the interactions between epithelial and stromal tissues. Other important factors are systemic conditions such as the hormonal status of the patient, the microenvironment of the malignancy and tumor-evoked immune responses.[10,11]

Prostate cancers usually present as androgen-dependent tumors, and androgen ablation is at present the treatment of choice, in particular for metastatic cancer. While this therapy can initially lead to substantial remissions, tumors frequently return in an androgen-independent, castration-resistant form that is highly resistant to further hormonal therapy and also to other available regimens, including chemotherapy. There is therefore a critical need for new, more effective treatments to improve disease management and patient survival. However, research in this area has been seriously hampered by a lack of clinically relevant, experimental *in vivo* models of the disease. While human prostate cancer xenografts in immunodeficient mice are generally considered to be most useful, the subcutaneous cell line xenograft models, commonly used for preclinical *in vivo* drug efficacy tests, do not adequately predict the efficacy of anticancer agents in the clinic.[12] Only about 5% of potential new anticancer drugs, that have successfully passed preclinical *in vivo* tests, have significant efficacy in clinical trials and are approved for clinical usage by the US Food and Drug Administration.[13] Experimental prostate cancer models with improved ability to predict clinical drug efficacy are therefore urgently required.

In developing clinically relevant human cancer xenograft models, displaying the various stages of prostate cancer, it appears essential to meet the following conditions: (i) use of a species of immunodeficient mice allowing high engraftment rates of all stages of the disease (localized and advanced forms), (ii) use of patient-derived specimens containing malignant tissue as well as adjacent benign tissue (e.g. tumor-associated fibroblasts) as part of the original three-dimensional architecture and microenvironment of the malignancy, (iii) use of a graft site enhancing retention of key characteristics of the cancers (e.g. tumor heterogeneity, genetic profiles) and (iv) a hormonal status of the host mimicking that of the patient. In adhering to these requirements, xenografts of a variety of low- to high-grade cancers (including prostate cancer) have been developed at the Living Tumor Laboratory (LTL; www.livingtumorlab.com) via subrenal capsule (SRC) grafting of patients' cancer tissues. To this end, nonobese diabetic/severe combined immunodeficient (NOD/SCID) or NOD/SCID IL2 receptor gamma chain null (NSG) mice were used. A high engraftment rate

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(~95%) has consistently been achieved, and at present, more than 170 transplantable cancer tissue xenograft lines (LTL series) have been established, stored frozen at various generations in a resurrectable form.[14–20] The SRC grafting methodology enhances retention of important properties of the patients' malignancies as indicated by retention of (i) tumor heterogeneity and androgen sensitivity,[15,18] (ii) tumor progression-related properties and suitability for predicting clinical drug responses for personalized chemotherapy[16,19] and (iii) genetic profiles and targeted drug sensitivity.[14,21,22] As such, SRC xenografting appears to be well-suited for development of cancer models with high clinical relevance.

This review deals mainly with experimental *in vivo* prostate cancer tissue xenograft models. Following a short overview of various types of prostate cancer models, it focuses on the development of patient-derived prostate cancer tissue xenograft models and their current and potential applications in preclinical studies.

OVERVIEW OF VARIOUS TYPES OF *IN VIVO* PROSTATE CANCER MODELS

In the last few decades various prostate cancer models have been developed. They include models of animal prostate cancer based on (i) spontaneous development of prostate tumors in aging dogs,[23] in rats,[24,25] and in genetically-engineered mice (GEM)[26] and (ii) transplantable, hormonally/chemically-induced carcinomas such as the Noble rat prostatic carcinoma.[27–29] Although such models contributed to the understanding of the development and progression of prostate cancer, they generally did not adequately predict the responses of human cancers to chemotherapy in the clinic.[30] This deficiency is thought to stem from significant differences between animal and human prostates in their anatomy and physiology, and from failure of the models to fully reflect the high complexity of human cancer biology.[31,32] Consequently, the focus of the research shifted toward use of human prostate cancer specimens that could survive, and grow in immunodeficient mice and be developed into transplantable tumor lines.

Cell line xenograft models

Classic models for human prostate cancer consists of immunodeficient mice carrying subcutaneous prostate cancer cell line xenografts generated by injection of cultured prostate cancer cells (e.g. LNCaP, PC3 or DU145) or coinjection of cultured prostate cancer cells and stromal cells. Such cell line xenograft models are valuable for basic studies, but unfortunately, have rather limited ability for predicting anticancer drug efficacy in the clinic.[33] This appears to be due to increased homogeneity of established prostate cancer cell lines after long-term *in vitro* culturing, contrasting with the heterogeneity of the parental cancers. Furthermore, cell line xenografts rarely possess the tissue architecture of the original cancer specimens from which the cell lines were derived, and consequently, do not accurately represent the complex biochemical and physical interactions between the cancer cells and various components of their microenvironment as found in the original malignancies.

Cancer tissue xenograft models

More realistic preclinical models for prostate cancer are thought to be provided by patient-derived cancer tissue xenograft models, based on direct implantation of fresh cancer tissue specimens into immunodeficient mice (e.g. nude, SCID mice). Such xenografts contain, especially initially, the cellular heterogeneity, architectural and molecular characteristics of the original cancer and its microenvironment.[34] However, successful grafting of cancer tissue is highly dependent on the type of graft site selected. Three graft sites in immunodeficient mice are mainly used, namely the subcutaneous, orthotopic and SRC sites. The subcutaneous graft site has various advantages, including easy implantation of the tissue and monitoring of the developing tumor using calipers, and hence is most commonly used. However, this site is known for its lack of vascularization and hence potentially inadequate nutrient supply that may lead to loss of cancer subpopulations, as indicated by low engraftment rates.[35] Furthermore, subcutaneous engraftment appears to be mainly successful when highly advanced cancers, e.g., metastatic and/or castration-resistant prostate cancers are used, representing only a small portion of the original cancer population.[36–38] While the orthotopic graft site provides a microenvironment similar to that of the original cancer and is theoretically the ideal graft site for testing spontaneous metastatic ability of prostate cancer tissue, the surgical procedure involved is quite challenging. In addition, the orthotopic site has a limited xenograft carrying capacity which severely restricts its use for establishing transplantable xenograft lines. Successful engraftment at the orthotopic site was found to be limited to highly advanced cancers, as found for the subcutaneous site. A different technical approach was therefore required for establishing both low- and high-grade human prostate cancer tissue xenografts allowing major retention of tumor heterogeneity. As described below, this is feasible by using the SRC graft site.

SRC GRAFTING OF PROSTATIC TISSUES

A major advantage of the SRC graft site is its provision of an instant blood supply due to the high vascularization of the kidney. The blood flow in this organ is very high and coupled to positive interstitial fluid pressure and a high rate of lymph flow.[39] Consequently, there is an exceptionally high fluid circulation within the extracellular space of the kidney.[40] This provides high graft perfusion, and the abundant supply of nutrients, hormones, growth factors and oxygen to transplanted cells and tissues (before they become vascularized) is likely instrumental to the success of the engraftment.[41–45] Access to the graft site is relatively easy via a small incision into the back of the host. Furthermore, the SRC site can accommodate tissues of quite a range of size and sources.[46]

Wang *et al.*[15] have compared grafting of normal human prostate tissues into the SRC, subcutaneous and orthotopic sites of immunodeficient mice and shown that the engraftment rate was 93.4% for the renal site, 58% for the subcutaneous site and 71.9% for the orthotopic site. A similar difference in the take rates of human prostate cancer tissues at these sites has been established by others.[47,48] It is evident from such comparisons that, of the three graft sites, the SRC site is most efficient for growing human prostate tumors as well as normal prostate cells. Furthermore, the greater vascularity of the renal graft site is associated with reduced selective pressure on the various cancer subpopulations present in the original heterogeneous primary tumor sample. Given the heterogeneity of cells within a primary prostate cancer, we postulate that the various cell types within the cancer vary significantly in their ability to tolerate the anoxia associated with the grafting process. For this reason, the more vascular renal graft site is very likely superior in preserving the original cellular complexity (heterogeneity) of the original primary tumor. This interpretation is supported by the high similarity observed between SRC xenografts and the parent tumors in histopathology, marker expression, genetic profiles and properties such as androgen sensitivity and metastatic ability.[14,15,47,48] These advantages of SRC xenografting indicate that this technique enhances maximization of

tumor engraftment rate as well as retention of the original cellular complexity of the primary tumor. Accordingly, cancer tissue lines developed at the SRC site should better reflect the wide spectrum of cancer cell types in the primary tumor than tumor tissue lines developed at the relatively anoxic subcutaneous site. Furthermore, once SRC tumor tissue lines are well established, they can be regrafted to, for example, the orthotopic site (the mouse prostate) for assessment of metastatic ability.

The SRC site has been used for some time for a variety of purposes, including growing embryonic or neonatal organ rudiments *in vivo* for extended periods, maintaining adult tissues *in vivo*, growing neoplastic cells and predictive testing of tumor response to chemotherapy in short term assays, e.g., the SRC assay in which the grafts are treated with anticancer drugs (for 6–11 days) right after transplantation of cancer tissue.[44,45,49,50]

More recently, SRC grafting has been used for establishing transplantable cancer xenografts. Such cancer tissue lines provide a valuable source of tumor tissue for studying various types of cancer. LTL transplantable prostate cancer tissue lines have been developed from patient's prostate cancer via SRC grafting and serial transplantation in NOD/SCID mice. They include lines which, to our knowledge, have been developed for the first time from prostate cancer biopsies, as well as lines developed from primary and metastatic tissues[20] (www.livingtumorlab.com). These transplantable tissue lines not only retain key biological properties of the original malignancies, e.g., histopathology, clinical markers expression and metastatic ability, but also chromosomal aberrations and gene expression profiles. They span various histopathological types of prostate cancer, e.g. adenocarcinoma and neuroendocrine prostate cancer (NEPC), as well as various molecular subtypes, encompassing diverse inter- and intratumoral heterogeneity. Furthermore, host castration led to the development of transplantable, castrate-resistant tumors, including the first model of complete neuroendocrine transdifferentiation.[20] It appears from the above that models based on such SRC xenografts more accurately mimic the malignancies in patients than conventional, cultured cell line-based models. As such the patient-derived cancer tissue xenograft models can be expected to be more clinically relevant and have greater predictability of drug efficacies in the clinic, and could be viewed as next-generation models. **Table 1** shows a comparison of the various properties of the major xenograft models.

APPLICATIONS OF NEXT GENERATION PROSTATE CANCER XENOGRAFT MODELS

The next generation xenograft models are useful for (i) fundamental prostate cancer research (e.g. identification of metastasis-related genes, new therapeutic targets), (ii) translational research (e.g. efficacy and toxicity testing of potential and established anticancer drugs, novel targeted therapeutic approaches) and (iii) personalized cancer therapy (**Figure 1**).

Fundamental prostate cancer research

The differences in growth rate, response to androgen ablation therapy and metastatic properties of sublines derived from a patient's specimen are very likely a reflection of the tumor heterogeneity of the original cancer. Therefore, prostate cancer tissue sublines displaying marked differences in specific biological and molecular characteristics are particularly useful for identification of novel biomarkers and/or therapeutic targets via comparative analysis. In our laboratory, a number of paired metastatic and nonmetastatic prostate cancer tissue sublines have been successfully developed from individual patients' primary cancer tissues, such as the paired metastatic PCa1-met and nonmetastatic PCa2 sublines,[51,52] the LTL220M and LTL220N sublines[18] and the LTL313B and LTL313H sublines.[53] By comparing gene and microRNA (miRNA) expression profiling of metastatic and nonmetastatic sublines derived from the same patient's prostate cancer specimens, molecular signatures of prostate cancer metastasis can be identified. Thus comparative serial analysis of gene expression (SAGE) of the paired metastatic PCa1-met and nonmetastatic PCa2 sublines led to identification of a novel gene, *ASAP1*, associated with prostate cancer metastasis. In clinical specimens, ASAP1 protein expression was found to be elevated in metastatic prostate cancer compared to primary cancers and benign prostate tissue. Functional studies indicated that the *ASAP1* gene plays an important role in prostate cancer cell migration and tissue invasion.[52] Similarly, we have utilized next generation sequencing to identify differentially expressed known and novel miRNAs in a pair of metastatic and nonmetastatic prostate cancer sublines, LTL313B and LTL313H, that likely include potential biomarkers for prostate cancer metastasis.[54]

Complex genomic rearrangements are frequently observed in cancer, but their impact on the tumor transcriptome is unknown. Sequencing the genomes and transcriptomes of the 313H xenograft model exhibited evidence of chromothripsis, a phenomenon leading to the simultaneous generation of tens to hundreds of genomic rearrangements. Several complex fusion transcripts, each containing sequences from three different genes, were identified.[55,56] These poly-gene fusion transcripts were expressed from chains of small genomic fragments originating from different parts of the genome that were recombined during a chromothriptic-type event. Furthermore, polygene fusion transcripts were detected in the prostate cancer cell line LNCaP, suggesting they may represent a common phenomenon. The implication that multigenic changes can give rise to polygene fusion transcripts is potentially of great significance to cancer genetics.

NEPC is an aggressive histopathological subtype of prostate cancer for which there is no effective therapy.[57] The cellular origin of NEPC and the molecular mechanisms involved in its development are largely unknown. Although findings based on clinical samples suggest that small cell neuroendocrine carcinoma may indeed develop from conventional adenocarcinoma via adaptation, no direct evidence for such a mechanism has been reported.[58,59] Recently, a complete transformation of adenocarcinoma (LTL331) to uniform NEPC (LTL331R) was observed after host castration. Importantly, both LTL331 and its castration-resistant counterpart, LTL331R, exhibited very similar chromosome copy number profiles, indicating an adaptive response rather than clonal selection.[20] This represents, for the first time, a capture of neuroendocrine transdifferentiation in a preclinical model, and provides strong evidence for epithelial plasticity. Therefore, this unique model of neuroendocrine transdifferentiation provides a valuable tool for studying hitherto unknown mechanisms of NEPC development and for developing novel therapeutic avenues.

Translational research

In the era of target therapy, it is important to evaluate drug efficacies using models showing clinically relevant expression of molecular targets. The next generation xenograft models developed by SRC grafting appear to provide a valuable platform for preclinical drug screening.

Models for reliable testing of anticancer drug efficacies are particularly important in case of aggressive malignancies, such as

Table 1: Comparison of major prostate cancer xenograft models

	Cell line xenograft	*Patient-derived PCa tissue xenograft (s.c.)*	*Patient-derived PCa tissue xenograft (SRC)*
Grafting site for transplantable line development	s.c.	s.c.	SRC
Parental tissue source	Metastatic tissue[a]	Metastatic tissue, few primary tissue	Biopsy, primary or metastatic tissue
Probability to establish a transplantable line	Low[b]	Low	High
Cancer cell origin	Human	Human	Human
Stroma cell origin	Mouse	Human, mouse (depends on generation)	Human, mouse (depends on generation)
Cancer heterogeneity	Relatively homogeneous	Relatively heterogeneous	Heterogeneous
Histology	Different from OT	Similar to OT	Highly similar to OT
Cancer cell karyotype	Highly different from OT	Similar to OT	Highly similar to OT

OT: original tumor; PCa: prostate cancer; s.c.: subcutaneous; SRC: subrenal capsule. [a]Followed by long-term *in vitro* culture, [b]Probability to establish cell lines from primary tissue is low

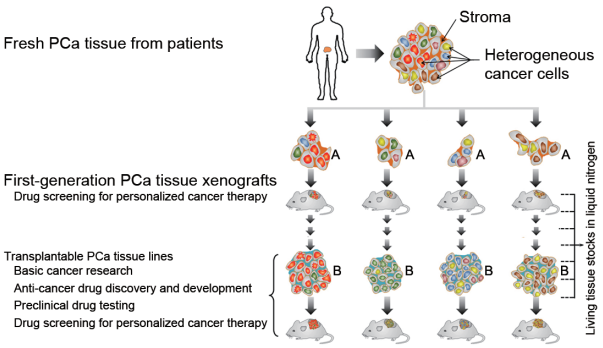

Figure 1: Diagram showing the development of next generation prostate cancer (PCa) tissue xenograft models and their applications. A, fresh PCa tissue from a patient containing heterogeneous cancer populations is cut into multiple pieces for immediate grafting under the kidney capsules of nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. B, transplantable PCa tissue lines are established via serial passages. The various cancer cell subpopulations are indicated by different colors. Human and mouse stroma are indicated in orange and blue, respectively. Transplantable cancer tissue lines can be preserved with 10% dimethyl sulfoxide in liquid nitrogen for long-term storage.

NEPC, for which a standard therapeutic regimen has not yet been adopted. Use of LTL352, a transplantable tissue line developed from a patient's NEPC specimen, has indicated that irinotecan, a topoisomerase I inhibitor, is potentially useful for therapy of refractory NEPC, in particular in combination with cisplatin.[60] Recently the LTL352 model was also used to determine the efficacy of a new aurora kinase inhibitor, PHA-739358, in NEPC.[61] Moreover, translational research using LTL313H, an androgen-dependent prostate cancer tissue line characterized by androgen receptor expression and serum prostate-specific antigen, has recently been instrumental in showing that regression of prostate cancer could be obtained by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor, EPI-001[53] and by using docetaxel in combination with Aneustat, a multivalent botanical drug candidate,[62] currently undergoing a phase I clinical trial (ClinicalTrials.gov Identifier: NCT01555242). We therefore anticipate that panels of such xenograft models, covering a number of molecular subtypes of the disease, will provide clinically relevant tools for the development of novel therapeutics for prostate cancer.

Personalized cancer therapy

Cancers generally consist of subpopulations of cells which can differ markedly in population size and sensitivity to specific treatments—differences in properties thought to underlie the varying responses of patients to a certain therapeutic regimen. Since each patient's cancer is unique, cancer therapy should ideally be tailored to individual patients. Choosing the most effective, least toxic and affordable chemotherapeutic regimen for a patient is one of the major challenges faced by oncologists today.[63] High toxicity of ineffective treatments could exclude a patient from undergoing alternative treatments. For predictive drug efficacy testing for personalized cancer therapy it is essential to use xenograft tissue that very closely mimics the patient's cancer and allows quick assessment of the patient's responses to a variety of regimens for subsequent implementation of the optimal regimen. For this purpose, first or early generation SRC xenografts of the patient's own malignancy may be useful. Such grafts likely feature most, if not all, of the molecular heterogeneity and histological complexity that exist in a patient's original cancer, contain stroma from the original tumor and mimic the cell-to-cell interactions of the patient's tumor microenvironment. The suitability of first generation xenografts for application in personalized cancer chemotherapy is indicated by studies of their suitability for predicting drug responses.[19,21,64]

An example of the potential usefulness of prostate cancer tissue lines for personalized chemotherapy has recently been obtained. Illumina genome sequencing of a patient's neuroendocrine prostate tumor showed a homozygous deletion on chromosome 9p21 spanning the 5'-deoxy-5'-methylthioadenosine phosphorylase (*MTAP*) and *CDKN2-ARF* genes, a common genetic deletion in various cancers. A xenograft line (LTL352), generated from the tumor, had the same genetic deletion as shown by Array Comparative Genomic Hybridization. Treatment of mice carrying the *MTAP*-deficient LTL352 xenografts with high doses of 6-thioguanine in combination with methylthioadenosine (to protect normal cells from 6-thioguanine toxicity), caused regression of the tumors while the hosts were not significantly affected by the treatment. This study demonstrates that use of appropriate, patient-derived cancer models in combination with advanced genomic profiling techniques can lead to identification of key therapeutic targets and therapies potentially useful for personalized oncology.[65]

CAVEATS AND POTENTIAL IMPROVEMENTS OF THE NEXT GENERATION XENOGRAFT MODELS

To obtain reproducible and reliable results with cancer tissue lines it is crucial that their

cellular characteristics and composition are maintained. Although following extended *in vivo* passaging, only minimal changes were observed in gross chromosome copy number, cell morphology, growth rates and gene expression profiles compared to early generation xenografts, it is prudent to establish a permanent stock of a xenograft line, at an early generation, ensuring that cellular characteristics and composition are preserved and avoiding alterations generated by continual passaging and unnecessary use of mice. Our studies indicate that cancer tissue lines can be preserved with 10% dimethyl sulfoxide in liquid nitrogen for long-term storage and can be successfully resurrected with a recovery rate of 95% when the SRC graft site is used.[20] Therefore, these xenograft tissue stocks can be used as a source of the original cancer tissue line and allow reproducible and reliable results.

A clear understanding of the molecular foundation of prostate cancer appears to be required for optimal assessment of the potential for disease progression. Recently, multiple molecular alterations, especially ETS and non-ETS gene rearrangements, have been identified in prostate cancer and may provide a rationale for molecular subclassification of the disease. The xenograft models derived from localized or metastatic prostate cancer tissues have provided valuable tools for studying various molecular alterations of the disease. It can be expected that a panel of such xenograft models, covering a number of molecular subtypes of the disease, will be useful for elucidating the functions of molecular alterations in prostate cancer progression and for developing novel therapeutic approaches for the disease.

CONCLUSIONS

Collectively, the panel of patient-derived prostate cancer tissue xenograft models, developed with a high success rate via SRC grafting of patients' cancer specimens into NOD/SCID mice, closely mimic the original cancers in terms of histopathology, tumor heterogeneity, chromosomal aberrations, gene expression profiles and tumor aggressiveness. As such, they can be viewed as next generation prostate cancer xenograft models that provide valuable tools with high clinical relevance for (i) studying the molecular and cellular development and progression of prostate cancer, (ii) developing new therapies and (iii) potential use for personalized therapy of the disease.

COMPETING INTERESTS

The authors declare no competing interests.

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