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SCIENCE DIRECT

Microvascular Research 69 (2005) 1–9

MVR Microvascular Research

www.elsevier.com/locate/ymvre

Novel syngeneic pseudo-orthotopic prostate cancer model: vascular, mitotic and apoptotic responses to castration

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Received 1 September 2004
Available online 15 December 2004

Abstract

We describe a novel syngeneic "pseudo-orthotopic" in vivo model of prostate cancer progression. Our model uses the dorsal skinfold chamber technique with fluorescence video microscopy and TRAMP-C2 tumor cells. The cells were transfected with a histone H2B-GFP fusion protein, permitting real-time measurement of tumor size, as well as mitotic and apoptotic indices. To generate a "pseudo-orthotopic" milieu, pieces of prostate tissue (10–15 mm^2) from donor mice were implanted into the chambers of C57BL/6 mice. The prostate tissue grafted into the chambers retained its native vasculature, as determined by transplantation of prostate tissue from GFP transgenic mice. TRAMP-C2 prostate cancer tumor spheroids (25,000 cells) were implanted in the chamber. Without prostate tissue, TRAMP-C2 prostate tumors were poorly angiogenic, displayed low mitotic and apoptotic indices (0.7×10^{-4}), and no significant tumor growth could be detected. TRAMP-C2 tumors growing on transplanted prostate tissue in the chamber on the other hand had mitotic indices in the order of 1.6×10^{-4} and apoptotic indices in the order of 0.8×10^{-4}. Furthermore, tumors with stroma were highly angiogenic, and were fully vascularized within 7–10 days. During a 4-week observation period, the number of tumor cells increased by nearly 300%. We used the model to study the effects of surgical castration. The most profound response was a rapid vascular regression of the tumor vasculature. Castration also increased apoptotic indices within the tumor without significant changes in mitosis. This model may be utilized for the rapid analysis of new therapeutic candidates against prostate cancer.

Keywords: Syngeneic; TRAMP; Animal model; Prostate cancer; Castration; Intravital microscopy

Introduction

Animal models are crucial to further our understanding of the mechanisms underlying the progression of prostate cancer. Unfortunately, the limited number of in vivo prostate tumor models has considerably hampered research. Research groups have expended tremendous effort into the development of human prostate tumor xenograft models (Stephenson et al., 1992; van Weerden and Romijn, 2000; Yang et al., 2001).

We previously evaluated the growth and angiogenesis of many human prostate tumor cell lines (DU145, PC3, and LnCAP) using intravital microscopy based on the dorsal skinfold chamber in nude mice (Frost et al., 2003). Poor growth (regression) of these cell lines in vivo as spheroids was attributed partly to the lack of appropriate tumor–mesenchymal interactions. Data also suggested, however, that the innate immune response was responsible for the inability of tumor xenografts to survive in the chamber system. These findings suggested against the use of a xenograph chamber model system for the study of micro-metastatic prostate cancer.

The TRAMP model (Transgenic Adenocarcinoma Mouse Prostate) is an attractive alternative to xenograft tumor models. TRAMP mice develop spontaneous prostate tumors with high frequency, due to expression of the SV40 large T-antigen under control of the prostate-specific rat probasin promoter (Greenberg et al., 1995). These mice develop a distinct pathology in the dorsolateral epithelium of the

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0026-2862/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.mvr.2004.10.001

prostate by 10 weeks of age. By 12 weeks, widespread infiltration of the prostate occurs, and by 24 weeks, they exhibit well-differentiated prostate tumors. By week 28, 100% of the animals have developed metastases in the lymph nodes and lungs (Gingrich et al., 1996). In parallel to human prostate patients, androgen ablation in TRAMP mice does not ultimately cure the disease due to the development of a hormone refractory state. Early castration in TRAMP model (12 weeks) (Gingrich et al., 1997) has a variable effect on progression of the disease and shows that prostate cancer is heterogeneous in this system. Although androgen ablation at 12 weeks can significantly decrease median primary prostate tumor burden, overall progression to poorly differentiated and metastatic prostate cancer is not ultimately delayed. Three carcinoma cell lines have been derived from a heterogeneous 32-week spontaneous tumor. Two of these three cell lines are tumorigenic in immune-competent syngeneic C57BL/6 hosts: TRAMP-C1 and TRAMP-C2 (Frost et al., 2003). TRAMP-C1 and TRAMP-C2 cells do not express in vitro and in vivo the T antigen oncoprotein, and are thus able to grow in nontransgenic, syngeneic C57BL/6 hosts (Foster et al., 1997). The establishment of syngeneic cell lines from the TRAMP transgenic mice provides a complete animal model system in which preclinical testing of new therapeutic approaches for prostate cancer can be investigated. A metastatic prostate model with TRAMP-C2 cells transplanted into C57BL/6 mice has been reported previously (Kwon et al., 1999). In this system, the TRAMP-C2 cell line developed metastases in the lymph nodes and lung from the primary tumor. Following primary tumor removal, all mice relapsed with metastases arising from micrometastases present at the time of the primary tumor resection.

As the disease in TRAMP mice mimics closely the evolution of human prostate cancer, this model offers a unique opportunity to test alternative approaches for the treatment of prostate cancer. The purpose of the present study was (a) to develop a syngeneic "pseudo-orthotopic" model for prostate cancer progression, and (b) examine the effects of castration using this model.

In our recent study, we provided data in support of the concept that tumor–mesenchymal interactions are of great importance in prostate cancer (Frost et al., 2003). With this in mind, we sought to introduce an orthotopic milieu to our system.

Methods

Animal model and surgical techniques

The dorsal skinfold chamber in the mouse was prepared as described previously (Lehr et al., 1993). Male mice (25–30 g body weight) were anesthetized (7.3 mg ketamine hydrochloride and 2.3 mg xylazine/100 g body weight, i.p.) and placed on a heating pad. Two symmetrical titanium frames were implanted into a dorsal skinfold, so as to sandwich the extended double layer of skin. A 15-mm full-thickness layer was then excised. The underlying muscle (M. cutaneous max.) and subcutaneous tissues were covered with a glass cover slip incorporated in one of the frames. After a recovery period of 1–3 days, tumor spheroids were carefully placed in the chamber.

Surgical castration

Mice were anesthetized as described above in surgical techniques. A lateral incision across the scrotum was made and the testes were individually removed, ligated, excised, and cauterized. The incision was then sutured and further sealed with Nexaband® acrylic.

Preparation of TRAMP-C2 prostate carcinoma cell spheroids

Tramp-C2 cells were transduced with a VSV pseudotyped LXRN virus encoding a Histone H2B-GFP fusion protein. The histone H2B-GFP cDNA was subcloned into the *Sal*I/*Hpa*I sites in the LXRN vector (Clontech, Palo Alto, CA) using *Sal*I and blunted *Not*I sites from the BOSH2BGFPN1 vector (Kanda et al., 1998). LH2BGFPRN virus was VSV pseudotyped in GP-293 cells pelleted by centrifugation and frozen at −80°C until use. Transduced Tramp-C2 cells were FACs sorted to generate a homogeneously labeled population. Tumor spheroids were generated with trypsinized TRAMP-C2 H2BGFP cells (250,000 tumor cells/ml) dispersed (100 μl/well) into 96-well round bottom plates coated with 1.0% agarose for a liquid overlay. The spheroids were allowed to compact for 48 h followed by washing in serum-free media for implantation on top of the prostate tissue (see below).

Grafting of stroma

Prostate tissue from a donor mouse was excised and minced into small pieces, followed by labeling with CMTMR for rhodamine counter-label for 15 min in serum-free media. The labeled prostate was then washed and implanted in dorsal skinfold chambers of C57BL/6 mice.

TUNEL assay

TrampC2 H2BGFP were seeded onto collagen-I-treated cover slips, at a density of 10,000/slip. The cells were treated with Cis-platinal (Bristol laboratories NDC 0015-3220-22) at 15 and 30 μM for 2 h. After the treatment, the cells were incubated for 30 h before they were fixed in 3% paraformaldehyde and permeabilized with 0.2% Triton-X. Apoptosis was detected by using a TACS TdT Kit (TA4625). Biotinylated nucleotides were incorporated into the 3′-OH ends of the DNA fragments by Terminal deoxynucleotidyl transferase (TdT). The biotinylated nucleotides were detected with streptavidin conjugated Alexa Flour 568 (red).