Dear Sir,

Orthotopic growth and metastasis of human prostate carcinoma in nude mice after transplantation of histologically intact tissue

Prostate cancer is the most frequently occurring tumor in men. Huggins and Hodges (1941) observed the androgen dependence of prostatic carcinoma growth, and since that time androgen ablation has been an important feature of therapy for prostate carcinoma. However, for prostate carcinomas that have become hormone-independent, there is no effective systemic treatment. Prostate cancer occurs in a high percentage of men over 50, but is aggressive in only a small percentage of patients. It is, therefore, necessary to develop models and markers to distinguish aggressive from non-aggressive tumors, and responsive from non-responsive ones, in order to make proper treatment decisions (Nakamura et al., 1992) and to develop new treatment strategies. A number of models for prostate cancer have been developed, including the Dunning R3327 hormone-dependent rat prostatic adenocarcinoma (Dunning, 1963). A number of cell lines from human prostatic carcinoma that grow in athymic nude mice, including LNCaP which is androgen-dependent (Horoszewicz et al., 1983), and the androgen-independent cell lines Du145 (Stone et al., 1978) and PC-3 (Kaighn et al., 1979; Koizumi et al., 1988) have also been isolated. Experimental in vivo growth of human prostate carcinoma lines has usually followed s.c. transplantation, with occasional metastatic activity, as first observed by Ware et al. (1982). Ware et al. (1985) were the first to demonstrate that differential injection sites affected the behavior of PC-3. Intraperitoneal injection (Sherwood et al., 1990) has been used and may result in higher metastatic activity. The PC-3 line has been injected into the tail vein of the nude mice while the inferior cava was occluded, which allowed tumor growth in the caval vein, kidneys, and lungs (Shevin et al., 1988). When PC-3 cells were injected into the peritoneal cavity, intra-abdominal growth resulted; when injected into the spleen, liver metastases resulted and when injected into the seminal vesicles, large tumors developed from there (Shevin et al., 1989).

More realistic models of human cancer can be constructed through orthotopic transplantation of the human tumor to the animal host (Wang et al., 1984). Recently we have developed orthotopic-transplantation techniques utilizing histologically intact tissue, including surgical specimens, to develop metastatic models in immunodeficient mice for human colon cancer (Fu et al., 1991a, 1992b), human bladder cancer (Fu et al., 1991b, Fu and Hoffman, 1992b), human lung cancer (Wang et al., 1992a,b,c; Kuo et al., 1992), and human pancreatic cancer (Fu et al., 1992a). For bladder cancer, at least, intact-tissue orthotopic transplantation resulted in greater metastatic expression than orthotopic transplantation of cell suspensions (Fu et al., 1991b; Fu and Hoffman, 1992). In this report we describe the results of orthotopic transplantation of histologically intact human prostatic carcinoma tissue.

Outbred nu/nu mice, 4–6 weeks old, were used for s.c. and orthotopic transplantation of Du145 and PC-3. All the mice were maintained in a pathogen-free environment. Cages, bedding, food and water were autoclaved and changed regularly. All the mice were maintained in a daily cycle of 12 hr light and 12 hr darkness.

Before orthotopic transplantation, each specimen harvested from s.c. growth in nude mice was inspected, and any grossly necrotic or suspected necrotic tumor tissue was removed. Each specimen was divided equally into 5 parts, each of which was subsequently cut into small fragments of about 1 mm³. Tumor fragments for each transplantation were taken from the 5 parts of each specimen in equal amounts.

Nude mice were anesthetized with isoflurane (Forane) inhalation, and iodine and alcohol swabs were used to sterilize the abdominal area. An incision was made along the midline of the lower abdomen. After proper exposure of the bladder and prostate, the capsule of the prostate was opened and 5–10 tumor fragments were inserted into the capsule. The capsule was then closed with an 8-0 surgical suture, and the abdomen was closed in one layer with 5-0 surgical sutures.

To confirm the human origin of the tumors growing in the nude mice, the Oncor total-human-genome probe (Oncor, Gaithersburg, MD) was used. Briefly, paraffin-embedded blocks of the nude-mouse tumor, both local and metastatic, were cut into 4-μm-thick sections and applied to silanized slides. After deparaffinization, protein digestion and dehydration, the Oncor biotinylated “Total-human DNA painting probe” was used for in situ hybridization. Avidin, anti-avidin antibody and horse-radish peroxidase-avidin complex with 3,3’-diaminobenzidine tetrahydrochloride (DAB) as the substrate was subsequently applied for the detection system according to the specifications supplied by Oncor. Hematoxylin was used in counterstaining. The nuclei of positive cells stained brown, indicating their human origin. Negative controls utilized the total procedure but without the human-genome-specific DNA probe. The human genomic probe was used to determine the human origin of the prostate tumors in the nude mice since neither Du145 nor PC-3 produce prostate-specific antigen (Gleave et al., 1992).

The human hormone-independent prostate-cancer line Du145 was grown subcutaneously in the nude mouse. The subcutaneously grown tumor was then resected and transplanted into nude mice orthotopically, as described above, using histologically intact tissue. Three mice were analyzed on days 71, 80 and 90, respectively, after orthotopic transplantation. Tumor growth was observed in all mice. The size of the primary tumor ranged between 1.7 cm³ and 2.1 cm³ (Fig. 1). The tumor appeared to be invasive and metastatic regionally.
The orthotopic transplantation resulted in local growth and metastasis to the bladder and kidney, as well as distant metastasis to the inguinal, iliac and mediastinal lymph nodes (Fig. 2). Hydronephrosis was observed due to urinary blockage by the locally-growing tumor. The human genomic probe demonstrated by in situ hybridization that the tumors were of human origin (Fig. 3) showing the positive hybridization stain for the tumor cells but not for the stroma or lymphocytes in the nude mouse lymph-node metastasis of PC-3. The histology of Du145 and PC-3 growing and metastasizing in the nude mice (Figs. 1, 2) matches published histologies of these tumors (Stone et al., 1978; Shevlin et al., 1988). This orthotopic transplant model of prostate carcinoma resembles the clinical picture of growth within the prostate capsule, showing urinary obstruction, hydronephrosis, local invasion, and distant metastasis. These models should offer opportunities for developing new therapeutic and diagnostic measures for prostate cancer, and may also be useful in predicting the clinical course of this disease for individual patients.

Yours sincerely,

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July 8, 1992.

REFERENCES


SHERWOOD, E., FORD, J., LEE, C. and KOZLOWSKI, J., Therapeutic efficacy of recombinant tumor necrosis factor alpha in an experimental


