Tissue vaccines for prevention and treatment of prostate cancer

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Abstract

Tissue vaccines are produced from harvested tumor material and contain a tremendously large menu of antigens, including those expressed only during in vivo growth and those associated with tumor stroma. Production of tissue vaccines from xenogeneic sources not only presents a means to overcome immunologic tolerance of tumors, but also represents a means to greatly expand availability of vaccine raw material compared to autologous vaccines. To determine if vaccination with tissue vaccines is a potential strategy for prevention and treatment of prostate cancer, we evaluated the utility of tissue vaccines derived from glutaraldehyde-fixed tumor (GFT) tissue and potassium thiocyanate extract (PTE) of tumor tissue. Subcutaneous tumors generated in Lobund-Wistar (LW) rats with the PAIII prostate cancer line were harvested, dissociated, and treated with 3% glutaraldehyde or prepared as an extract with potassium thiocyanate. To evaluate prevention of prostate cancer, groups of thirty LW rats were treated with intravenous methylnitrosourea (30 mg/kg) to induce autochthonous prostate tumors and were then vaccinated subcutaneously (SC) monthly from 2 to 10 months of age with either media (MEM), GFT or PTE. At 12 months of age, gross and histological examination of prostates showed 50% and 90% reductions in the incidence of prostate cancer in PTE- and GFT-vaccinated rats, respectively, compared to media-vaccinated controls (Fig. 1). Using the same model, groups of rats underwent weekly vaccination with MEM or GFT when palpable tumors were first detected in the caudal abdomen to evaluate the utility of tissue vaccines as a treatment for prostate cancer. Though size of the primary tumor was not significantly reduced, 70% of GFT-vaccinated rats were free of metastasis compared to only 10% of controls (Fig. 2). To determine if this vaccine could be used as a xenogeneic preparation for human prostate cancer, immunocompetent Ncr-Foxn1\textsuperscript{nu} mice were vaccinated SC with the GFT vaccine; their splenocytes harvested 7 days after the last boost and co-incubated with human PC346 prostate cancer cells (Group 1); and orthotopically transplanted into syngeneic BALB/c nu/nu mice. Groups of 20 nu/nu mice were treated this way or with PC346 cells co-incubated with splenocytes from media-vaccinated mice (Group 2); or transplanted with untreated PC346 cells (Group 3). Ten weeks later, the mice were euthanized and prostates evaluated for tumor growth. The incidence of prostate cancer was reduced by 70% in Group 1 mice compared to those in Groups 2 and 3, indicating that this vaccine has xenogeneic efficacy. In summary, tissue vaccines represent a means to harvest numerous powerful and novel antigens which cannot be captured in any other way, and which can be effectively used to prevent and treat prostate cancer.

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1. Main Text

Cancer of the prostate gland is the most commonly diagnosed cancer in men and the second most common cancer resulting in death of men, on an age-adjusted basis. In the majority of cases, prostate carcinoma is a disease of older men, many of whom have comorbid conditions which elevate the risk of cancer death. The possibility that the patient’s own immune system might be stimulated in such a way as to effectively combat cancer has come under renewed interest recently. Though the very existence of a tumor suggests failure to generate an effective immune response, a number of studies provide evidence that vaccination is a safe and effective means to both prevent and treat some cancers.

An emerging body of evidence shows that dynamic epithelial–stromal interactions in solid tumors may select subsets of stromal cells with the ability to modulate tumor behavior, and the local microenvironment promotes emergence of tumor-associated stromal cells with functions different from the normal stroma. For example, fibroblasts derived from breast tumors stimulated morphogenesis and growth of breast preneoplastic epithelial cells, while fibroblasts derived from normal breast tissue inhibited this process. Such functional changes in tumor stroma may partly be derived from the changes in secretion of growth factors and in the extracellular matrix. In any case, cancer-associated fibroblasts are functionally and phenotypically distinct from normal fibroblasts.

Beyond the neoplastic cells within a tumor, the connective tissue stroma represents an enormously unexploited reservoir of potentially powerful antigens for cancer immunotherapy. Indeed, in some carcinomas, the stromal compartment may account for up to 90% of the tumor mass. Tissue vaccines are constructed directly from harvested tumor material, thus including not only cancer cells but connective tissue stroma as well. A major difficulty with cancer vaccination has been the genetic and phenotypic plasticity of many tumors. Through a number of mechanisms, cancer cells can develop means to escape immunosurveillance and destruction (1-3). In contrast to cancer cells, however, tumor stroma cells are genetically more stable and should therefore represent targets which are less able to escape destruction by the immune system (4). Further, because many tumor stroma-associated antigens are up-regulated or expressed only in the tumor microenvironment, they represent highly unique moieties which are unlikely to be recognized as ‘self’ antigens. Against this backdrop, vaccines created from harvested tissue, including stroma, create an opportunity to overcome problems associated with immunotolerance and lack of sufficient antigenic choice (5). Because they are composed of material directly harvested from tumors, an additional advantage of tissue vaccines is that they include antigens expressed following in vivo growth versus the more limited antigenic profile of cultured cells.

To determine if vaccination with tissue vaccines is a potential strategy for prevention and treatment of prostate cancer, we evaluated the utility of tissue vaccines derived from glutaraldehyde-fixed tumor (GFT) tissue and potassium thiocyanate extract (PTE) of tumor tissue. PAIII prostate adenocarcinoma cells were used to generate tumor tissue from which vaccines were prepared. This cell line was originally isolated at the Lobund Institute of the University of Notre Dame from an autochthonous, metastatic prostate adenocarcinoma in a LW rat. Cells were grown in Modified Eagle’s medium (MEM) at 37°C in a CO₂ incubator, and harvested by mechanical disruption after 72 h of growth. PAIII cells were implanted subcutaneously into the flanks of 2-3 month old male LW rats and tumors harvested 21 days later.

Two tissue vaccine preparations were evaluated: a glutaraldehyde-fixed tumor (GFT) tissue vaccine; and a potassium thiocyanate (KSCN) extract (PTE) tissue vaccine. The GFT vaccine was produced by incubating for 60 minutes in 2.5% glutaraldehyde, followed by extensive washing, tumor tissue dissociated by passage through an 80-mesh screen. The PTE vaccine was prepared by lysis of harvested tumor tissue with 1 M KSCN and subsequent dialysis for washing.

To demonstrate the ability of tissue vaccines to prevent autochthonous cancer, groups of 30 LW rats were vaccinated monthly from age 2 to 10 months with either modified Eagle’s medium (MEM; control), GFT, or PTE. Methylnitrosourea (MNU) was administered intravenously at age 4 months. At 12 months of age, gross and histological examination of prostates showed 50% and 90% reductions in the incidence of prostate cancer in PTE- and GFT-vaccinated rats, respectively, compared to media-vaccinated controls.

To demonstrate the ability of a tissue vaccine to inhibit metastasis from a primary tumor, groups of rats were treated with MNU to induce tumorigenesis. When palpable tumors were present in the prostate, rats were randomly divided into groups vaccinated with either MEM or the GFT tissue vaccine. Rats were vaccinated weekly until euthanasia due to declining clinical condition or at 8 weeks following initial vaccination, whichever came sooner.
Though size of the primary tumor was not significantly reduced, 70% of GFT-vaccinated rats were free of metastasis compared to only 10% of controls.

To determine if the GFT tissue vaccine can be used as a xenogeneic preparation for human prostate cancer, immunocompetent Ncr-Foxn1<nu> mice were vaccinated SC with the GFT vaccine; their splenocytes harvested 7 days after the last boost and co-incubated with human PC346 prostate cancer cells (Group 1); and orthotopically transplanted into syngeneic BALB/c nu/nu mice. Groups of 20 nu/nu mice were treated this way or with PC346 cells co-incubated with splenocytes from media-vaccinated mice (Group 2); or transplanted with untreated PC346 cells (Group 3). Ten weeks later, the mice were euthanized and prostates evaluated for tumor growth. The incidence of prostate cancer was reduced by 70% in Group 1 mice compared to those in Groups 2 and 3, indicating that this vaccine has xenogeneic efficacy. Further, evaluation of supernatants of cultured splenocytes from vaccinated immunocompetent mice showed significant increases in amounts of TNF-α, IL-2, IFN-γ, and IL-12, cytokines associated with Th1 immunity.

In summary, tissue vaccines represent a means to harvest numerous powerful and novel antigens which cannot be captured in any other way, and which can be effectively used to prevent and treat prostate cancer. Tissue vaccines stimulate Th1 immunity and can be produced from a xenogeneic source.

References