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Prophylactic vaccines for prevention of prostate cancer

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1. ABSTRACT

Prostate cancer is the most prevalent cancer and the second leading cause of cancer death in men. There are various modalities for treatment of prostate cancer. Immunotherapy with several vaccines and antibodies has also been successfully used with positive clinical outcome in prostate cancer patients. The majority of these vaccines are palliative and have been employed when a person is already diagnosed with prostate cancer. The aim of this article is to review various vaccines that have been examined for immunoprophylactic prevention of initiation/development/metastasis of prostate cancer. The Pubmed database and Google Scholar search identified 26 articles on various vaccines that have been investigated for prophylactic prevention of cancer development. These vaccines targeted prostate-specific/restricted antigens (PSA/PSMA/PSCA), oncoproteins (GRP/MUC family, erbB2/HER-2/*neu*), whole tumor cell antigens, prostate regulating hormones (GnRH/testosterone), and various cytokines and immune modulators. The data indicates that the development of immunoprophylactic vaccines for prostate cancer is an exciting proposition, which can translate into a viable reality for clinical application in humans.

2. INTRODUCTION

Prostate cancer is the most prevalent cancer in the Western male population and the second leading cause of cancer death in men, affecting over 10 million individuals. It is primarily detected by measuring prostate-specific antigen (PSA) in serum (1-3). PSA is prostate specific, however not prostate cancer specific, any abnormality of prostate can raise PSA levels. Treatment modalities for prostate cancer include radiation therapy (brachytherapy/external beam radiation therapy/proton beam radiation therapy), radical prostatectomy, androgen deprivation therapy, chemotherapy and cryotherapy. Patients with metastatic prostate cancer have an overall approximate 23% survival rate over 5 years. Immunotherapy has also been successfully used with positive clinical outcome in prostate cancer patients of various stages. In the 1970's immunotherapy of prostate cancer using Bacillus Calmette Guerin (BCG) was examined, followed by in the 1990's with vaccine against gonadotropin-releasing hormone (GnRH) that demonstrated some degree of success (4-6). It was followed by phase I/II/III clinical trials in prostate cancer patients using various other molecules that illustrated successful outcome. Although several of these vaccines have showed promising,

Table 1. Various vaccines that are immunoprotective against tumor cell challenge

Vaccine	Species	Method	Results	Ref.
1. Vaccines based upon prostate-specific/restricted antigens				
A. Prostate specific antigen (PSA)				
1. Mycobacterium tuberculosis hsp 70-HPV E7 antigen-PSA	C57B1/6(H-2^b) mice	Mice immunized with the vaccine Tumor challenge one week after last immunization	Vaccinated mice were better protected against tumor challenge PSA-specific CTLs were generated	7
2. pVax-PSA	C57BL/6 (H-2^b) mice	Mice immunized with pVax-PSA plus plasmids (pcDNA3-mGM-CSF and/or pNGVL1-mIL-2) Tumor challenge ten days after vaccination	pVax-PSA vaccine alone protected 40% of mice With either co-adjuvants 60% tumor protection When both adjuvants co-administered, 80% tumor protection	8
3. pPSA	BALB/c mice and Cynomolgus monkeys	Mice immunized with the vaccine Tumor challenge one week after last vaccination	Mice immunized with pPSA showed significant tumor protection up to 100%	9
4. pPSA	C57BL/6 mice	Mice immunized with three different regimens Tumor challenge one week after last vaccination	Delay in time of tumor appearance, retarded tumor growth, and prolonged survival	10
5. Ad5-PSA (type 5 adenovirus) with recombinant canarypox virus (ALVAC) encoding IL-12 and TNF-α	BALB/c mice	Mice immunized with Ad5-PSA with or without ALVAC Challenged with RM11 prostate cancer cells expressing PSA	Vaccine with cytokine deliveries were protected against tumor challenge	11
6. Ad5-PSA with CpG ODN	BALB/c (H-2^d) mice	Mice immunized with vaccine Tumor challenge with RM11 prostate cancer cells expressing PSA	Vaccine with CpG improved life expectancy, protected against tumor challenge	12
7. pPSA and pIL-18	BALB/c mice	Mice immunized with vaccine Tumor challenge one week after last immunization	Mice immunized with pPSA alone showed 100% protection at higher doses. Vaccine including pIL-18 even the low doses also showed 100% tumor protection	13
B. Prostate-specific membrane antigen (PSMA)				
8.a. Proteosome (tVacs;hPSMAt) b. Secreted proteins (sVacs;hPSMAs)	Copenhagen rats	Rats immunized with vaccines with or without rGM-CSF Tumor challenge two weeks after last immunization	100% protection of rats immunized with vaccine including GM-CSF	14
9. NIH3T3 cells cotransfected with pST/neo plus pEF-BOS based vectors expressing full-length PSMA or C-terminal region	BALB/c mice	Mice immunized with the vaccine Tumor challenge one week after last immunization	Survival of tumor bearing mice significantly increased and tumor growth decreased by vaccination	15
10. Gene therapy through transduction of PSA or PSMA into dendritic cells (DCs)	BALB/c mice	Mice were injected with modified DCs Tumor challenge 1-18 weeks after last injection	Gene modified DC recipients had increased protection from specific tumor challenge for at least 18 weeks post-vaccination DC vaccination protected both male and female recipients against tumor cell challenge	16
C. Prostate stem cell antigen (PSCA)				
11.a. pCIneo-mPSCA b. pCIneo-mSTEAP1	TRAMP mice	Mice immunized with either vaccine Tumor challenge ten days after last immunization	91% immunized mice protected against tumor but GU tract weight was significantly reduced	17
12. mSTEAP	C57BL/6 and MLR/lpr mice	Mice immunized with vaccine Tumor challenge ten days after final immunization	Mice survival significantly prolonged due to protection from tumor challenge	18
2. Vaccines based upon oncoproteins				
A. Gastrin-releasing peptide (GRP)				
13. GRP_{18-27}(GRP6)	C57BL/6 mice	Mice immunized with vaccine Tumor challenge ten days after final immunization	Mice immunized with vaccine showed significant inhibition of tumor growth	19

B. Various glycoprotein and carbohydrate antigens				
14. Tn(c)-KLH-QS21	Human males with biochemically relapsed prostate cancer	Patients immunized with the vaccine	40% of patients remained active and free of disease for 55 months and PSA levels dropped	20
15. a. MUC-2-KLH b. Globo H glycolipid-KLH	Human males	Patients immunized with either vaccine	Vaccines found to be safe and raised high titer antibodies Immunoprotective effect of antibodies not examined.	21
C. erbB2/HER-2/*neu* antigen				
16. erbB2tr-lentivirus	C57BL/c and BALB/c mice	Mice immunized with vaccine Tumor challenge six weeks after last immunization	Immunized mice demonstrated up to 100% tumor protection	22
17. HER2/neu peptide (E75)	High-risk prostate cancer (HRPC) patients that had undergone prostatectomy	HLA-A2+ patients immunized with vaccine	HER2/neu (E75) prevented or delayed recurrences in HRPC patients if vaccinated before PSA recurrence	23
3. Vaccines based upon whole tumor cell antigens				
18. ALVAC-IL-2/IL-12/TNF-α	C57BL/6 mice	RM-1 cells were isolated from tumors, infected with ALVAC-IL-2/IL-12/TNF-α, mice were immunized and then challenged with tumor cells	100% tumor protection	24
19. B16-Flt3L (Fl3vax) coupled with CTLA-4 blockade	C57BL/6 mice	Mice immunized with vaccine Tumor challenge	Combination of Fl3vax with CTLA-4 promoted rejection of established TRAMP prostate adenocarinomas	25
20. TAg-positive SV40-transformed cells combined with systemic recombinant IL-12	C57BL/6 TRAMP mice	Mice immunized with vaccine Tumor challenge	Prostate carcinogenesis significantly delayed by vaccination, but death occurred 5-6 weeks after onset of tumor	26
21. Mycobacterium vaccae bacilli (SRL172) with whole allogenic tumor cells LyLu and PAIII)	Copenhagen and Lobund-Wistar rats	Rats immunized with vaccine Tumor challenge	80% of Copenhagen immunized rats and 40% of Lobund-Wistar rats were tumor protected	27
22. Whole tumor cells expressing HSVtk/ganciclovir danger signals	Lobund-Wistar rats	Rats immunized with vaccine Tumor challenge one week after immunization	Both autologous and allogeneic vaccinations showed significantly slower tumor growth and enhanced survival rate	28
23. Whole tumor cells expressing CD/5-FC suicide gene	Lobund-Wistar rats	Rats immunized with vaccine Tumor challenge one week after final vaccination	Delay of tumor growth and enhanced survival rate	29
24. Whole tumor cell vaccine combined with docetaxel drug	C57BL/6 mice	Mice were given docetaxel with whole tumor cell vaccine Tumor challenge	Combination of docetaxel and vaccine induced protection in >80% of mice	30
4. Vaccines based upon prostate-regulating hormones				
A. Gonadotropin-releasing hormone (GnRH)				
25.a. GnRH3-hinge-MVP in adjuvant b. GnRH3-hinge-MVP-Hsp65	C57BL/6 mice	Mice immunized with either vaccine Tumor challenge three weeks after first immunization	Both vaccines induced GnRH-specific antibodies, significant survival observed especially with second vaccine, tumor size and weight decreased significantly and gonadotropins levels decreased	31
5. Vaccines based upon immune cell antigens				
26. AdCD40L	TRAMP-C2 mouse	Mice immunized with vaccine Tumor challenge one week after final vaccination	Dose-dependent protection, 83% of immunized mice tumor free	32

successful results, these are palliative and are employed when a person is already diagnosed with prostate cancer. There are only a few studies that have investigated whether or not vaccination can prevent the initiation/development/metastasis of prostate cancer.

The aim of this article is to review different vaccines that have been examined for immunoprophylactic prevention of initiation/development/metastasis of prostate cancer. Several laboratories are actively involved in identifying prostate-specific/restricted genes/proteins that can be used for early diagnosis and development of immunoprophylactic vaccines. The Pubmed database (www.pubmed.gov) and Google Scholar (http://scholar.google.com) were searched (2000-2011) using the keywords: “Prostate Cancer Vaccines” AND “Prophylactic Prostate Cancer Vaccines.” The Pubmed database, identified 651 articles using the former and 21

articles using the later keywords. Google Scholar search identified 16,700 articles for "Prostate Cancer Vaccines" and 7,280 articles for "Prophylactic Prostate Cancer Vaccines" keywords. Further analysis identified 26 articles focusing on vaccines targeting several antigens for the prophylactic prevention of prostate cancer development. Most of these studies have used an active immunization approach, but a few have also examined the passive immunotherapeutic strategies using preformed antibodies. These vaccines can be broadly divided into five categories (Table 1) and are described below.

3. DISCUSSION

Both synthetic/recombinant peptides and DNA vaccines have been examined for prostate cancer prevention. DNA vaccines have several distinct advantages including easy manipulation, use of a generic technology, simplicity of manufacture, and chemical/biological stability. In general, DNA vaccines induce Th1 response and the response can be modulated using various growth factors/cytokines/chemokines/CpG motifs.

3.1. Vaccines based upon prostate-specific/restricted antigens

3.1.1. Prostate-specific antigen (PSA)

One of the critical factors in vaccine design is selection of an antigen. Of all the molecules, PSA is the most obvious target for vaccine development. PSA is expressed in prostate epithelial cells, has a highly restricted tissue distribution, and 95% of patients with prostatic malignancies express PSA. Although PSA is a self-antigen, T cell responses to PSA epitopes have been detected in men and prostate cancer patients. Mice do not express PSA in their prostates. Male monkeys express a closely related gene. Several studies have examined the effect of vaccination against PSA with or without co-stimulatory molecules. Most of these studies have yielded positive results. Pavelenko *et al* examined the efficacy of DNA vaccine based upon PSA (7). Heat shock proteins (Hsp) increase immunopotency of DNA-based vaccines. Several plasmid based vectors encoding chimeric proteins containing PSA, fused to various Hsp's, were generated and mice were immunized with these constructs. Although the vaccination induced CD8+ cytotoxic lymphocytes (CTLs) to specific PSA, it did not provide protection against the challenge with PSA expressing tumor cells. It seems that Th2 antibody response is required to neutralize PSA for immunoprotection. In another study (8), a DNA vaccine expressing PSA with or without combination with plasmids coding for granulocyte macrophage-colony stimulating factor (GM-CSF) and/or interleukin-2 (IL-2) were constructed and used for immunization. The PSA DNA vaccine co-injected with IL-2 and GM-CSF protected 80% of mice against a PSA-expressing tumor challenge. Another study investigated male mice and cynomolgus monkeys immunized with a PSA DNA vaccine. Both developed PSA-specific antibodies and mice also were immunoprotected against challenge with PSA expressing tumor cells (9). Ahmad *et al* constructed a DNA vaccine expressing human PSA gene (10). Male mice were injected via intra-muscular (i.m.) electroporation with the DNA vaccine. One week after the last vaccination, mice were challenged subcutaneously (s.c.) with transgenic adenocarcinoma of the mouse prostate cancer (TRAMP)C1/hPSA and tumor growth was monitored. Serum from animals were examined for anti-hPSA antibodies and interferon (IFN)-gamma. The phPSA vaccine therapy significantly delayed the appearance of tumors and resulted in prolonged survival of the animals. Immune responses were tumor specific and were transferable in adoptive T cell transfer experiments. Another vaccine, the recombinant PSA adenovirus type 5 (AD5-PSA), was constructed and was found to be safe and effective in inducing expansion of CD8+ T cells with enhanced CTL activity against the antigen-bearing tumor cells *in vitro*, as well as *in vivo* in a mouse model of prostate cancer (11). The AD5-PSA vaccine combined with cytokine gene delivery is not only immunoprotective, but also eliminates large established tumors that are refractory to other interventional methods. Mice immunized with the combined approach of Ad5-PSA and CpG enhances protection against the subsequent tumor challenge, as compared to mice immunized with the vaccine alone (12). Another study examined the effect of PSA DNA vaccine co-administered with pIL-18 plasmid (13). Low doses of the pPSA vaccine were not capable of inducing tumor protection, but when pIL-18 was co-administered, complete tumor protection was observed in all mice. Tumor protection was mediated by both CD4+ and CD8+ T cells.

3.1.2. Prostate-specific membrane antigen (PSMA)

The prostate-specific membrane antigen (PSMA; gamma carboxyl peptidase II (GCPII)), has a restricted tissue expression and is expressed only in the prostate gland, brain tissue, jeunum and proximal kidney tubules. Its expression is increased approximately 10-fold in prostate cancer cells. Elevated PSMA serum levels have been observed in healthy men and women, as well as in patients with benign prostate hypertrophy, prostate and breast cancer. Two plasmid DNA vaccines, encoding either products that are retained in the cytosol and degraded in the proteasome (tVacs, hPSMAt), or secreted proteins (sVacs; hSPMAs), were evaluated for stimulation of cytotoxic cell or antibody responses (14). The *in vivo* immunization with both tVacs and sVacs leads to generation of cytotoxic T cells. Co-administration of GM-CSF during immunization enhances the immune response and immune animals are protected against development of tumors when tumor cells expressing the target antigen are inoculated. In another study, mice were vaccinated with NIH3T3 cells co-transfected with pST/neo plus pEF-BOS-based vectors expressing either the full-length 750-amino acid human PSMA or only the C-terminal 180-amino acid region (PSMc) (15). PSMc lies C-terminal to the transferrin receptor-like sequence in the extracellular domain of PSMA. The mice were injected intraperitoneally (i.p.) 4 times at weekly intervals with vaccine cells. Vaccinated mice were then challenged s.c. with Renca/PSMA; a BALB/c renal cell carcinoma line transfected to express human PSMA. Growth of Renca/PSMA tumors was substantially retarded and host survival significantly prolonged in mice pre-vaccinated

with either 3T3/PSMA or 3T3/PSMc. The antitumor activity induced by vaccination with 3T3/PSMc, was also demonstrated via growth inhibition of established LNCaP tumors xenografted in athymic mice following passive transfer of immune serum from vaccinated mice.

The effect of gene therapy using PSA and PSMA has also been investigated (16). Gene therapy for prostate cancer may be realized through transduction of genes into immunotherapeutic dendritic cells (DCs). An oncoretroviral vector encoding human PSMA and a bicistronic oncorectroviral vector encoding human PSA and cell surface CD25 cDNAs were constructed. *In vitro*, transduced DCs retained allostimulatory function and primed syngeneic T cells for tumor antigen-specific IFN-gamma secretion. In test experiments designed to elucidate mechanisms *in vivo*, syngeneic recipients of transduced DCs had increased anti-human PSA antibody titers and tumor-specific CD8+ T cell IFN-gamma secretion with no detectable immune response to CD25. Gene modified DC recipients had increased protection from specific tumor challenge for at least 18 weeks post-vaccination. DC vaccination also protected both male and female recipients. Gene-modified DC vaccination mediated regression of established, specific gene expressing, TRAMP-C1 prostate cancer cell tumors.

3.1.3. Prostate stem cell antigen (PSCA)

Prostate stem cell antigen (PSCA) is a cell surface protein whose biological function is not clear. It is a member of the Ly-6 family and is involved in signal transduction and cell-cell adhesion. PCSA is overexpressed in 80-90% of primary and 100% of bone-metastatic prostate cancer tissue. It is expressed in both the androgen-dependent and androgen-independent prostate cancer, and increased levels of PSCA correlate with higher Gleason score, advanced stage and the progression to androgen independence. Another highly expressed protein is six transmembrane epithelial antigen of the prostate 1 (STEAP1). Its function is currently unknown, however it seems to act as a channel or transporter protein. STEAP1 is highly expressed at all stages of prostate cancer and is also present in other tumors. It shows a restricted expression in normal human tissues. The murine and human PSCA and STEAP1 have high homology at both nucleotide and amino acid sequences. Two antigen delivery platforms, recombinant DNA and modified vaccinia virus Ankara (MVA) vectors, both encoding either mPSCA or mSTEAP1 were used in diversified DNA prime/MVA boost vaccination protocol (17). Antitumor activity was evaluated in TRAMP-C1 s.c. syngeneic tumor model and TRAMP mice. DNA prime/MVA boost immunization against either mPSCA or mSTEAP1, delayed tumor growth in TRAMP-C1 cells-challenged mice. The simultaneous vaccination with both antigens produced a stronger anti-tumor effect against TRAMP-C1 tumors, than vaccination with either mPSCA or mSTEAP1 alone. Most importantly, concurrent DNA prime/MVA boost vaccination regimen with those antigens, significantly decreased primary tumor burden in TRAMP mice without producing any apparent adverse effects. Active immunization against PSCA and STEAP1, using DNA prime/MVA boost strategy, is a promising immunoprophylactic approach for prevention and/or treatment of prostate cancer. Similar results were found in another study that used mSTEAP (18). This study found that mSTEAP-based vaccination was able to induce a specific CD8+ T cell response against mSTEAP that prolonged the overall survival rate in tumor-challenged mice.

3.2. Vaccines based upon oncoproteins

3.2.1. Gastrin-releasing peptide (GRP)

Gastrin-releasing peptide (GRP), a bombesin-like peptide, can act as a growth factor in many types of cancer and has been regarded as a molecular target for anticancer therapy. A DNA vaccine using a plasmid vector to deliver the immunogen of six copies of the B cell epitope GRP_{18-27} (GRP6) was constructed (19). In order to increase the potency of this DNA vaccine, multiple strategies were applied including DNA-prime protein-boost immunization and introduction of a foreign T-helper epitope into DNA vaccine. Mice vaccinated with DNA vaccine and then boosted with HSP65-GRP6 protein induced high antibody titers and relatively high avidity of anti-GRP antibodies, that caused an inhibition of the growth of murine prostate carcinoma.

3.2.2. Various glycoprotein and carbohydrate antigens

Synthetic glycoprotein (MUC-1, MUC-2) and carbohydrate (Globo H, GM-2, Tn (c), TF (c)) antigens when conjugated to the carrier, keyhole limpet hemocyanin (KLH), and mixed with the saponin adjuvant, QS21, can elicit high titer IgM and IgG antibodies with specificity for the immunizing molecule in patients with biochemically relapsed prostate cancer (20). GPI-0100 is a semi-synthetic saponin with modifications designed to augment stability and diminish toxicity. Including GPI-0100 for vaccination, decreases the potential toxicity of the vaccine (21).

3.2.3. erbB2/HER-2/*neu* antigen

Gene therapy strategies may accelerate the development of prophylactic immunotherapy against cancer. A lentiviral (LV) vector encoding a kinase-deficient form of erbB2 (erbB2tr) was used to transduce murine DCs (22). Murine erbB2 is a clinically relevant tumor-associated self antigen; its human homolog (HER-2/*neu*) is overexpressed in breast cancer and in 80% of metastatic prostate cancers. Following one infection, approximately 47% of DCs overexpressed erbB2tr. To determine whether low doses of transduced DCs could protect mice from prostate cancer cells, prime/boost vaccination with erbB2tr-transduced DCs was performed. Six weeks after vaccination, mice were simultaneously bilaterally challenged with the aggressive RM-1 prostate cancer cell line and an erbB2tr-expressing variant (RM-1-erbB2tr). Whereas control mice developed both tumors, all recipients of erbB2tr-transduced DCs demonstrated erbB2tr-specific tumor protection. Protection against RM-1-erbB2tr tumors was associated with sustained levels of anti-erbB2tr antibody production and also correlated with erbB2tr-specific Th1 cytokine secretion.

E75 is an immunogenic peptide from the HER2/*neu* protein that is expressed in prostate cancer.

High-risk prostate cancer (HRPC) patients, demonstrating varying levels of HER2/*neu* expression, were vaccinated with E75 peptide plus GM-CSF to prevent postprostatectomy PSA and clinical recurrences (23). The findings indicate that the HER2/*neu* (E75) vaccine can prevent or delay recurrence in HRPC patients, if completed before PSA recurrence. A larger randomized phase II trial is required to confirm these findings.

3.3. Vaccines based upon whole tumor cell antigens

Human prostate cancers characteristically express low levels of major histocompatibility complex (MHC) Class I, which makes it challenging to induce protective antitumor responses involving T cells. Whole cell tumor vaccines can induce protective T cell immunity to low MHC Class I-expressing mouse prostate cancer cell line, RM-1 (24). ALVAC recombinant canarypox viruses encoding IL-2, IL-12, and tumor necrosis factor (TNF)-alpha can be used to create therapeutic vaccines in two different ways. The RM-1 cells can be pre-infected *in vitro* with the viruses prior to injection (pre-infection vaccine) or the RM-1 cells can be injected alone, followed by the viruses (separate injection vaccine). The pre-infection vaccine resulted in 100% clearance of primary tumors, whereas intradermal (i.d.) delivery of the separate injection vaccine cleared 40-60% of primary tumors. Despite the highly efficient primary tumor clearance by the pre-infection vaccine, only the separate injection vaccine generated protection upon rechallenge. Tumor-free survival induced by the separate injection vaccine required natural killer (NK) cells, CD4+, and CD8+ T cells.

Irradiated tumor cells can be used as a vaccine also. However, the induced antitumor responses are ineffective due to lack of immune costimulation. GM-CSF is a strong adjuvant for transforming irradiated tumor vaccines into mediators of tumor protection and sometimes rejection. GM-CSF enhances this transformation by inducing differentiation and maturation of antigen presenting cells (APCs) such as DCs and by chemoattracting granulocytes, macrophages, and lymphocytes to the vaccine. Like GM-CSF, the cytokine Fms-like tyrosine kinase 3 ligand (Flt3L) expressed in B16 and coupled with CTLA-4 blockade, promotes both prophylactic and therapeutic rejection of B16. When administered at the site of the growing tumor, Gvax fails to prevent tumor outgrowth in mice, whereas the B16-Flt3L vaccine (Fl3vax) induces the rejection of 75% of melanomas implanted 3 day pre-vaccination. Relative to Gvax, Fl3vax promotes greater infiltration of CD8+ T cells and sentinel/plasmacytoid DCs at the vaccine and tumor sites. Gvax and Fl3vax did not synergize when used in combination in treating B16 melanoma, even in the context of CD25+ regulatory T cell depletion. The combination of Flt3L expression and CTLA-4 blockade can also promote the rejection of established TRAMP prostate adenocarcinoma (25).

Active immunization combining several specific antigens and adjuvants can enhance the immunoprotection (26). A vaccine consisting of allogeneic large T antigen (TAg)-positive SV40-transformed cells combined with systemic recombinant IL-12 was administered to TRAMP mice, starting from when they were still tumor-free at 5-6 weeks of age. The combined vaccine significantly inhibited prostate carcinogenesis, giving a more than doubled median latency time of prostatic tumors. Vaccination with cells or IL-12 treatment alone was poorly effective. The combined vaccine induced a very high CD4+ response biased toward the Th1 pathway, with the induction of a humoral response that included TAg-specific antibodies. The active immunoprophylactic approach based on the combination of allogeneic SV40 TAg-positive cells and systemic administration of recombinant IL-12, significantly delayed autochthonous urogenital carcinogenesis driven by SV40 TAg.

In another study, to examine the immunoprophylactic effect of whole allogeneic tumor cell vaccines, two rat prostate cancer models were used (27). MAT-LyLu tumors which grow in Copenhagen rats and PAIII tumors which grow in Lobund-Wistar rats were crossed over to test the effect of allogeneic vaccination. Irradiated tumor cells were administered as s.c. vaccines either before tumor challenge or after tumor establishment. A preparation of heat-killed Mycobacterium vaccae bacilli (SRL172) was used as an adjuvant to increase vaccine efficiency. Prophylactic vaccination with allogeneic MAT-LyLu cells protected against PAIII tumor challenge in Lobound-Wistar rats, with 80% of animals surviving for more than five months, compared with 40% for animals receiving autologous cells. The immunity was prolonged, as rats were protected when rechallenged 5 months later. In Copenhagen rats, allogeneic PAIII cells protected against the more aggressive MAT-LyLu tumor challenge only when the cells were combined with SRL172. These experiments are interesting and indicate that allogeneic vaccines are more immunogenic and immunoprotective.

Destruction of tumor *in vivo* by genetic prodrug activation therapy leads to a marked local and systemic immune response, local T cell infiltration and the establishment of T-cell immunity. It is postulated that immunostimulation may be due to induction of danger signals and the inherent immunogenicity of products of HSVtk/ganciclovir kill. Triphosphorylated ganciclovir is genotoxic and a potent recombogenic and chromosomal-break-inducing agent. The efficacy of anti-tumor vaccines comprising irradiated allogeneic or autologous whole cells expressing HSV/tk, which are first killed *in vitro* by prodrug activation using ganciclovir, were evaluated (28). The vaccination was found to be effective in both models and superior to traditional irradiated whole tumor cells even after single doses. Protection against tumor challenge was associated with marked proliferative and Th1 cytokine responses. The same group confirmed these findings in another study, indicating that the immunogenicity of irradiated tumor cells is enhanced when they are killed *ex-vivo* using suicide-gene therapy (29). This approach would be applicable clinically in terms of ease of vaccine production, safety, storage and avoidance of potential toxicities of *in vivo* gene transfer.

Taxanes are used as cancer chemotherapeutic agents. Docetaxel is a member of the taxanes family and is widely used to treat various cancers including prostate cancer. The combination of docetaxel and tumor cell vaccine enhances the antitumor effects by inhibiting tumor

growth (30). This study indicated that potency of the vaccine, can be enhanced by combining various chemotherapeutic agents that modulate immune cells.

3.4. Vaccines based upon prostate-regulating hormones

3.4.1. Gonadotropin-releasing hormone (GnRH)

Gonadotropin-releasing hormone (GnRH) is normally synthesized by hypothalamic neurons and secreted into hypophysioportal circulation via portal vessels. It selectively stimulates gonadotropin cells to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thus playing a central role in the neuroendocrine regulation of reproduction. Besides the main role of GnRH in the regulation of pituitary gonadotropes, GnRH is now widely accepted as a multifunctional neuropeptide. GnRH-specific binding sites have been reported in a number of human tumors, such as prostate and breast cancers. Among the various approaches investigated in controlling GnRH activity, the active immunization using peptide-based vaccines is exciting. Induction of anti-GnRH antibodies might thus become beneficial in treatment of such hormone-dependent diseases. GnRH vaccines could suppress concentration of LH and testosterone in peripheral circulation and cause atrophy of testes in numerous species, thus achieving the treatment of androgen-dependent prostate carcinoma. GnRH immunization also causes atrophy of prostate and other accessory sex glands in rats and bonnet monkeys (5). A chimer peptide of GnRH3-hinge-MVP, containing three copies of GnRH (GnRH3), hinge region of human IgG and T helper epitope of measles virus fusion protein (MVP), can induce an immune response against GnRH. However, immunization with GnRH vaccines requires strong adjuvants to enhance the response. Recently, Hsp65 was reported as a carrier molecule, linked to peptide, for production of antibodies against peptide. GnRH2-hinge-MVP of conjugation to Hsp65, was used as an adjuvant-free vaccine to assess the therapeutic effect of GnRH immunoneutralization on tumor development in the mouse model (31). Compared with mice treated with Hsp65 and PBS, mice of the orthotopic model receiving *in situ* treatment, GnRH3-hinge-MVP-Hsp65 showed a significant prolongation of survival and suppression of local tumor growth. Serum levels of both testosterone and LH were reduced by treatment with GnRH3-hinge-MVP-Hsp65. The conjugation of the recombinant GnRH peptide to Hsp65 or other carrier proteins could provide a promising approach for the development of an efficacious vaccine against the prostate cancer.

3.5. Vaccines based upon immune cell antigens

CD40 is a member of the TNF receptor superfamily. It is expressed on a variety of cell types, including B-lymphocytes, macrophages, DCs, endothelial cells, fibroblasts, and epithelial cells. The interaction of CD40 with its ligand (CD40L, CD154) is critical in promoting both humoral and cellular immune responses. In addition to the direct anti-proliferative and proapoptotic effects of CD40 ligation in carcinoma cells, the influence of CTLs may provide further therapeutic opportunities. Adenovirus (AD)CD40L-transduced TRAMP-C2 cells were used in prophylactic vaccination studies, while therapeutic studies were performed using peritumoral injections of ADCD40L (32). ADC40L yielded reduced TRAMP-C2 cell viability and induced apoptosis *in vitro*. Vaccination with CD40L-expressing TRAMP-C2 cells induced anti-tumor immunity and peritumoral ADCD40L injections induced tumor growth suppression.

4. CONCLUSIONS

The development of immunoprophylactic vaccines for prostate cancer is an exciting proposition. The recent data discussed above in various animal models and humans indicate that this proposition can translate into clinically viable reality for application in humans. Besides several unexplored antigens (33, 34), the prostate-specific/restricted antigens (PSA/PSMA/PSCA), oncoproteins (GRP/MUC family/erbB2/HER-2/*neu*), whole tumor cell antigens, prostate-regulating hormones (GnRH/testosterone), and various cytokines and immune modulators can provide ideal targets for development of immunoprophylactic vaccines. Although the prostate-specific/restricted/regulators can be utilized specifically for prostate cancer immunointerception, other molecules can also be used for prevention of other cancers. Vaccines based upon GRP has been examined for melanoma and breast cancer (35, 36), Flt3-ligand for melanoma (37), HSVtk/ganciclovir for glioma (38), HER-2/*neu* for breast cancer (39), and suicidal gene therapy (CD-5FC) for melanoma (40). In conclusion, vaccination is a viable approach for prevention of cancer initiation, development, metastasis and growth. Like vaccines developed against various bacterial and viral infections, which have demonstrated tremendous success to almost worldwide eradication of several diseases, the development of immunoprophylactic vaccination for cancer is definitely needed.

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